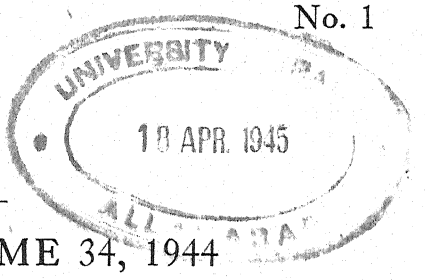
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ERRATA, VOLUME XXXIV

Pages 10 and 11, table 1, *read Milium for Millium* and *Puccinellia for Puccinielia*

Page 13, last line, *read vulgare for vulare*

Page 17, paragraph 2, line 13, *read* grass *for* gross

Page 18, paragraph 4, *read Milium for Millium*

Page 71, line 10, *read* from below the tip *for* from the tip

Page 137, paragraph 2, lines 11 and 12, *read* 10 per cent copper and 90 per cent sulphur *for* 70 per cent copper and 30 per cent sulphur

Page 249, line following footnote 6 should follow line 5 of text

Page 335, in title, in paragraph 1, line 2, and in paragraph 3, line 1, *read Levisticum officinale for Ligusticum scoticum*

Page 340, paragraph 2, line 1, *read Levisticum officinale for Ligusticum scoticum*

Pages 359 and 362. The legend under map on page 359 belongs to diagram on page 362 and the legend under diagram on page 362 belongs to map on page 359

Page 372, line 3, *read* 2 L *for* 1.4 L

Page 402, line 20, *read* materials not eliminated *for* materials eliminated

Page 509, last line, *read* propiono *for* propriono

Page 583, line 4, *read* 4576–1 *for* 4567–1

Page 606, line 1, *read Daucus carota for Daucus carotae*

Page 628, paragraph 3, line 3, and page 630, paragraph 3, line 9: *after* no menace (page 628) and *after* when planted (page 630) *add* aside from the possibility of introducing the fungus into the soil

Page 727, line 16, *read sprengeri for sprengleri*

Page 773, transpose diagrams of figures 1 and 2

Page 950, line 6, *read* Oestland *for* Pestlund

Page 990, paragraph 3, *read* W. J. Zaumeyer, President; H. B. Humphrey, Vice-President; *for* H. B. Humphrey, President; W. J. Zaumeyer, Vice-President

Page 992, *read* C. L. LEFEBVRE, *for* C. L. LEFABVRE

SPECIALIZATION OF PATHOGENICITY IN ERYSIPHE GRAMINIS ON WILD AND CULTIVATED GRASSES[1]

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INTRODUCTION

Since the discovery of pathogenic specialization in *Erysiphe graminis* DC. by Marchal (12), many authors have contributed to our knowledge of the subject. Seven physiologic varieties of the fungus have been described on the basis of restriction of infection to the species of a given genus of grasses. Some of these physiologic varieties have been further subdivided into physiologic races distinguished by their ability to infect certain species or agronomic varieties of species within a host genus. Powdery mildew of the cereals has been most comprehensively studied, but in most instances only a few grasses were tested in host range studies. Blumer (1) lists the pathogen on 76 species of 32 genera of the Gramineae, but cultures of the fungus from only 7 genera have been studied intensively. The present investigation was undertaken primarily to determine the infection range of the fungus from other grasses.

LITERATURE REVIEW

The first cross-inoculation experiments with the Erysiphaceae were reported by Wolff (33). His inoculation of species of *Lupinus* and *Trifolium,* host plants of *Erysiphe communis* (*E. polygoni*), with *E. graminis,* and inoculation of grasses with *E. communis* gave negative results, according to Salmon (22). Wolff (32), however, reported successful infection of wheat with ascospores of *Erysiphe graminis* from perithecia formed on wheat.

Marchal (12) reported the first positive infection from cross-inoculation experiments with *Erysiphe graminis,* and, on the basis of host specialization, distinguished the following 7 specialized forms: *tritici* on species of *Triticum, hordei* on species of *Hordeum, secalis* on species of *Secale, avenae* on species of *Avena* and *Arrhenatherum elatius, poae* on species of *Poa, agropyri* on *Agropyron* species, and *bromi* on species of *Bromus.*

Erysiphe graminis tritici, as distinguished by Marchal, infected *Triticum polonicum, T. spelta, T. turgidum* but not *Agropyron* spp., *Avena sativa,*

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Bromus spp., *Hordeum vulgare, Poa* spp., *Secale cereale, Triticum dicoccum, T. durum,* or *T. monococcum.* Marchal (13) reported further that ascospores produced in perithecia obtained from *Triticum vulgare* infected *T. vulgare* but not *Agropyron caninum, Avena sativa, Hordeum vulgare,* or *Secale cereale.*

Marchal's results were confirmed by Salmon (23), who states that wheat mildew could not infect *Agropyron repens, Avena sativa, Hordeum vulgare,* or *Secale cereale.* Salmon (28) transferred wheat mildew to young plants of *Hordeum silvaticum.* Reed (16) pointed out that the *Index Kewensis* lists *Hordeum silvaticum* as a synonym of *Elymus europaeus.* Reed further states that plants resulting from a wheat × rye hybrid were highly resistant to *Erysiphe graminis tritici* and *E. graminis secalis.* Information on the reaction of wheat varieties is given by Reed (17), and, quoting from unpublished results (18), he states that wheat mildew infected different species of *Aegilops.*

Reed (19) transferred wheat mildew to *Aegilops aristata, A. aucheri, A. cylindrica, A. ligustica, A. speltoides, A. squarrosa* L., *A. triaristata, Triticum dicoccum* Schrank., *T. durum* Desf., *T. freycenetii* Host, *T. meyeri, T. monococcum* L., *T. polonicum* L., *T. spelta* L., *T. thaoudar* Reut., *T. tumonia* Schrad, *T. turgidum* L., and *T. vulgare* Vill. Both resistant and susceptible varieties were found in the *Triticum* species. There was no infection of *Aegilops ovata* L., *A. triticoides, Avena sativa* L., *Brachypodium distachyum* Beauv., *Hordeum vulgare* L., *Secale cereale* L., or *Triticum dicoccum.*

Mains (10) distinguished 2 physiologic races within *Erysiphe graminis tritici,* and discussed the reactions of many varieties of several species of *Triticum.* The following grasses proved very resistant to physiologic race 1: *Agrostis alba* L., *A. perennans* (Walt.) Tuckerm., *Agropyron cristatum* J. Gaert., *A. repens* (L.) Beauv., *Arrhenatherum elatius* (L.) Beauv., *Bromus ciliatus* L., *B. erectus* Huds., *B. hordeaceus* L., *B. japonicus* Thunb., *B. mollis* L., *B. pumpellianus* Schribn., *B. secalinus* L., *B. tectorum* L., *Festuca elatior* L., *F. rubra* L., *Elymus canadensis* L., *E. condensatus* Presl., *E. striatus* Willd., *E. virginicus* L., *Hordeum jubatum* L., *H. murinum* L., *H. nodosum* L., *H. pusillum* Nutt., *Hystrix hystrix* Millsp., *Secale montanum* Guss., and *Sitanion hystrix* (Nutt.) J. C. Smith. Also to physiologic race 1, resistant and susceptible varieties were reported in *Triticum vulgare* Vill., *T. spelta* L., *T. polonicum* L., *T. compactum* Host, *T. durum* Desf., *T. turgidum* L., *T. dicoccum* Schrk., *T. stramineum,* and only resistant varieties were found in *T. monococcum* L., *T. dicoccoides, T. persicum,* and *T. timopheevi.* To physiologic race 2 *Aegilops squarrosa* L., and *A. ventricosa* Tausch. were moderately resistant, *A. crassa* Boiss., *A. cylindrica* Host, *A. ovata* L. and *A. triuncialis* L. were very resistant, and several strains of *Secale cereale* and *S. montanum* exhibited no signs of infection.

Schlicting (29) distinguished 6 races of *Erysiphe graminis tritici* in Germany and another race that in all probability is undescribed. These

differ from the two races of Mains. Rosenthiel (21) reported 5 races of *E. graminis tritici* in Germany, but the differential varieties of Schlicting were not tested.

Marchal (12) found that *Erysiphe graminis hordei* infected *Hordeum hexastichon, H. jubatum, H. murinum, H. nudum, H. trifurcatum, H. vulgare* and *H. zeocriton,* but not *H. bulbosum, H. maritimum,* or *H. secalinum.* Also there was no infection of *Agropyron caninum, A. giganteum, A. repens, Agrostis alba, Aira caryophyllea, Alopecurus pratensis, Andropogon ischaemon, Anthoxanthum odoratum, Arrhenatherum elatius, Avena fatua, A. orientalis, A. sativa, Brachypodium sylvaticum, Briza media, Bromus arduennensis, B. macrostachys, B. mollis, B. patulus, B. racemosus, B. secalinus, B. sterilis, B. squarrosus, Cynosurus cristatus, Dactylis glomerata, Deschampsia flexuosa, Elymus arenarius, Festuca elatior, F. gigantea, F. rubra, Holcus lanatus, Koehleria cristata, Lolium perenne, Melica ciliata, Milium effusum, Phleum boehmeri, P. pratense, Poa annua, P. mulalensis, P. nemoralis, P. pratensis, P. serotina, P. trivalis, Secale cereale, Setaria viridis, Trisetum flavescens, Triticum polonicum, T. spelta,* or *T. vulgare.*

Marchal (13) states that ascospores from perithecia collected on barley were able to develop on *Hordeum distichum, H. trifurcatum, H. vulgare,* and *H. zeocriton.*

Salmon (24) reported that ascospores from barley produced no infection on *Avena sativa, Secale cereale* or *Triticum vulgare.* With conidia from *Hordeum vulgare* Salmon (25) infected *Hordeum decepiens, H. distichon, H. hexastichon, H. intermedium, H. vulgare* and *H. zeocriton.* A slight infection was obtained on *H. bulbosum* and *H. maritimum,* but no infection was produced on *Avena sativa, Hordeum jubatum, H. murinum, H. secalinum, H. sylvaticum, Triticum vulgare* or *Secale cereale.*

Reed (16), by inoculation with conidia from *Hordeum vulgare,* produced infection on *Hordeum distichon* L., *H. nudum* L., *H. steudelii* × *trifurcatum, H. tetrastichon* L., and young seedlings of *H. nodosum* L. Negative results were obtained on *Avena sativa* L., *Hordeum bulbosum* L., *H. maritimum* With., *H. jubatum* L., *Triticum dicoccum* Schrank., *T. durum* Desf., *T. vulgare* Vill. and *Secale cereale* L.

Montemartini (14) could not infect unspecified species of *Avena, Holcus,* or *Setaria* with conidia from *Hordeum murinum.*

Mains and Dietz (11) found resistant and susceptible varieties in *Hordeum deficiens, H. distichon, H. intermedium,* and *H. vulgare.* On the basis of host specialization they distinguished 5 physiologic races. In continuation of this line of investigation Tidd (30) described 2 additional races, i.e., races 6 and 7.

Honecker (4, 5, 6, 7) has described 9 races of *Erysiphe graminis hordei* for Germany. Comparison with American work is impossible, because Honecker did not use the same differential varieties of barley.

Erysiphe graminis secalis was described by Marchal (12) as infecting *Secale cereale* and *S. anatolicum* but not *Avena sativa, Hordeum vulgare* or *Triticum vulgare.* Also, Marchal inferred that there was no infection on

species of *Agropyron, Bromus* or *Poa.* Marchal (13) reported that ascospores of *E. graminis secalis* infected only *Secale cereale.*

Reed (15) states that conidia from *Secale cereale* infected *S. anatolicum, S. cereale,* and *S. montanum* but not *Avena sativa, Bromus mollis, Dactylis glomerata, Festuca elatior, F. heterophylla, Glyceria fluitans, Hordeum vulgare, H. jubatum, Lolium perenne, Phleum pratense, Poa compressa, P. pratensis, P. trivialis, P. nemoralis, Secale dalmaticum,* or *Triticum vulgare.*

Treboux (31) reported that conidia from *Secale cereale* infected *S. cereale* but not *Hordeum vulgare* or *Triticum vulgare.*

Mains (9) discovered several strains of *Secale cereale* that were resistant to *Erysiphe graminis secalis.*

Erysiphe graminis agropyri is described by Marchal (12) as occurring on species of *Agropyron.* Conidia from *Agropyron giganteum* and *A. repens* would not infect barley. This specialized variety would not infect *Avena sativa, Bromus* spp., *Hordeum vulgare, Poa* spp., *Secale cereale* or *Triticum vulgare.*

Salmon (27) states that conidia from *Agropyron repens* infected *A. caninum, A. repens,* and *A. tenerum,* but there was no infection of either *A. acutum* or *A. glaucum.*

Comparable results were obtained with *Erysiphe graminis avenae* by Marchal (12), Salmon (23, 27), and Reed (19, 20), with *E. graminis poae* by Marchal (12), Salmon (27), and Reed (15), with *E. graminis bromi* by Marchal (12) and Salmon (23, 26), and *Erysiphe graminis* from *Dactylis glomerata* by Salmon (27). Marchal (12) states that *Erysiphe graminis* from *Holcus lanatus* and *Festuca pratensis* would not infect *Hordeum vulgare.*

The literature records to date indicate that the physiologic varieties of *Erysiphe graminis* are mostly restricted to the species of one genus. The only exceptions have been slight infections by *Erysiphe graminis tritici* on *Hordeum sylvaticum* (*Elymus europaeus*) by Salmon (28) and on species of *Aegilops* by Reed (19), slight infection of *Arrhenatherum elatius* by *Erysiphe graminis avenae* by Marchal (12) and Reed (18) and slight infection of *Hordeum sylvaticum* (*Elymus europaeus*) by *Erysiphe graminis hordei* by Salmon (25). The following results indicate that this concept of extremely narrow specialization is mostly erroneous.

MATERIALS AND METHODS

Three hundred seventy-two accessions of the grass and cereal species[3] listed in table 1 were used. Fifty to 100 seeds were planted in 3½-inch pots containing a mixture consisting of 1 part peat, 1 part sand, 3 to 4 parts sterilized soil and a small amount of commercial fertilizer. The plants thus obtained were maintained in a separate greenhouse free from mildew and formed a stock supply of seedlings. Since the grass species vary in rate of growth, this method is necessary in order that seedlings of all species be

[3] Hitchcock (2) was followed in the classification of the grass species where possible.

available for inoculation at a given time. Some grass species must be replanted occasionally to provide a constant supply of young seedlings. For inoculation with a mildew culture 5 to 25 seedlings of each grass were transplanted to 2½-inch pots. Such sets were transferred to separate greenhouse compartments where cultures of the fungus were maintained.

The scattering or dilution method described by Mains (8) and Tidd (30) was used to obtain highly pure and probably, in most cases, single-spore cultures. This purification was done after a knowledge of pathogenicity of the bulk culture was obtained.

The writer employed the following method of inoculation: In order that the spores may not be dispersed by air currents, the plants on which a mildew culture is being maintained are placed for 24 to 48 hours in a glass-covered chamber (8). Under these conditions a large quantity of conidia accumulates. The inoculum is collected at midday by lightly tapping the leaves, allowing the masses of conidia to fall into the lower half of a Petri dish. The spores are then floated on water and poured into a 250-cc. flask. The amount of water added is estimated by the number of plants to be inoculated. The aqueous spore suspension in the flask is thoroughly agitated and applied by an atomizer. Many spores are deposited in each water droplet on the leaves. The plants are then covered for 24 hours with wet muslin cloth supported on a wire frame. This method has given uniformly good results. All leaves of all plants can be inoculated easily. On susceptible grasses 6 to 8 days after inoculation numerous infection areas are produced. It has the further advantage of not distributing spores into the air to the extent produced by the dusting method. The leaf areas resulting from new growth developed between the inoculation and recording of notes show no infection. Cloths, atomizers, and glassware used in inoculations were sterilized between cultures.

During the course of the investigation results in variance with those of previous workers were obtained with certain cultures. These results were verified under conditions that exclude the possibility of foreign contamination in the following manner. Seeds of the plants in question were planted in 3½-inch pots filled with the soil mixture described above. Glass lamp chimneys with the top end covered by two layers of light muslin-cloth were then pressed down a short distance into the soil inside the pot and the seedlings grown inside the lamp chimneys. These glass covers (8) were removed only when inoculations were performed, and then only for a few seconds while conidia were transferred by a scalpel to the leaves of plants to be inoculated. These plants were grown in a separate greenhouse free from mildew-infected plants, and the inoculum was brought into the isolated culture house in a covered Petri dish.

Notes were taken 7 to 10 days and 14 to 18 days after inoculation. Two readings proved desirable, since the inoculation sometimes produces considerable initial infection of a higher degree than will persist. The conidial suspension method provides a very heavy inoculation, and it is nearly impossible for a grass to escape the inoculation.

The five types of reaction of Mains and Dietz (11, p. 231) were employed. The symbols + and – were used to further indicate the degree of susceptibility.

Field observations of the reaction of seedling and adult plants of the grass accessions were found useful in the selection of mildew cultures for study. Many of the grasses used in this study are represented in the observational row nurseries of the Soil Conservation Service at Pullman, Washington. During the summer months of 1939, 1940, and 1941 at Pullman, the writer recorded observations on mildew reaction of many of the grasses being studied. From these data it was possible to select, efficiently, mildew cultures for study and to obtain a better understanding of greenhouse tests. A grass pathology nursery including all of the grass accessions being studied was maintained at the Botanical Garden of the University of Michigan. This nursery proved to be an excellent source for mildew cultures and for mildew observations. Mildew cultures were purposely selected for study which appeared to be different by field observations.

RESULTS

In table 1 are summarized the results of inoculations of grasses and cereals with 8 cultures of *Erysiphe graminis*. Details of significant results with each culture are discussed below.

Culture 18. On *Secale cereale*, from Yakima, Wash.

Culture 18 was almost completely restricted to *Secale cereale*. The only other infection was a very resistant reaction on *Agropyron spicatum*. The infection results reported for many grass species represents the first large scale, negative evidence yet presented for the fungus from rye.

Culture 2. On *Triticum aestivum*, from Ann Arbor, Mich.

This culture produced very unusual results. Infection was produced on a number of grass species of several genera, all in the tribe Hordeae. A very susceptible reaction was recorded on *Aegilops crassa, A. cylindrica, Agropyron striatum, Elymus condensatus* and *E. junceus*, and a moderately susceptible reaction was exhibited on *Aegilops triuncialis, Agropyron inerme, A. spicatum, Elymus canadensis, E. dahuricus, E. triticoides, E. glaucus, E. sibiricus* and *Sitanion hystrix*. Also, a moderately resistant reaction was recorded on *Agropyron spicatum, Elymus glaucus* and *Sitanion jubatum*. Such a wide infection range for wheat mildew is in direct contrast to all previous records, since *Aegilops* embraces the only species outside the genus *Triticum* that have been reported infected by wheat mildew (10, 19).

Culture 2 does not differ from physiologic race 1 of Mains (10) on the basis of the following reactions of two differential wheat varieties: very susceptible—Malakoff C.I. 4898, and highly resistant—Axminster C.I. 1839. Mains reported the reactions of the largest number of grasses to wheat mildew prior to the present study, and, except for a few species of *Aegilops*,

TABLE 1.—*Summary of infection experiments with accessions of grasses and cereals tested to eight cultures of Erysiphe graminis*

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
Aegilops crassa Boiss.	1[a]	1	.....	2	.....	.....	.....	.....	2	.....	.....	2	.....	.....	.....	.....
A. cylindrica Host	1	.....	.....	1	1	.....	.....	.....	.....	1	.....	1	.....	1	.....	.....
A. triuncialis L.	1	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....
Agropyron caninum (L.) Beauv.	.....	1	.....	1	.....	.....	.....	1	.....	1	.....	.....	.....	.....	.....	.....
A. cristatum (L.) Beauv.	.....	4	.....	4	3	.....	.....	4	1	3	.....	4	3	1	.....	1
A. desertorum (Fisch.) Schult.	.....	1	.....	1	1	.....	.....	1	1	.....	1	.....	.....	1	.....	1
A. inerme (Scribn. and Smith) Rydb.	1	7	.....	12	12	.....	.....	11	11	.....	10	1	9	1	.....	4
A. intermedium (Host) Beauv.	.....	1	1	.....	1	.....	.....	1	1	.....	.....	.....	.....	.....	.....	1
A. repens (L.) Beauv.	.....	1	.....	1	2	.....	1	1	1	.....	1	.....	1	.....	.....	1
A. semicostatum (Steud.) Nees	.....	1	.....	1	1	.....	.....	1	1	.....	1	.....	1	.....	.....	1
A. sibiricum (Willd.) Beauv.	.....	5	.....	5	5	.....	.....	5	4	1	2	3	2	3	.....	.....
A. smithii Rydb.	.....	1	.....	1	1	.....	.....	1	.....	1	1	.....	1	.....	.....	.....
A. spicatum (Pursh) Scribn. and Smith	2	3	1	1	13	.....	.....	14	8	2	11	2	5	1	1	.....
A. striatum (Steud.) Nees ex Hook.	1	.....	1	.....	1	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....
A. subsecundum (Link) Hitchc.	.....	1	.....	1	1	.....	.....	1	1	11	.....	.....	1	.....	.....	.....
A. trachycaulum (Link) Malte	.....	14	1	13	13	.....	1	13	3	.....	6	8	4	7	.....	3
Agrostis alba L.	.....	4	.....	4	.....	4	.....	4	.....	4	.....	4	.....	4	.....	4
A. exarata Trin.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
A. hiemalis (Walt.) B.S.P.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
A. interrupta L.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
A. palustris Huds.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
A. scabra Willd.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
A. spica-venti L.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
A. stolonifera L.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
Alopecurus aequalis Sobol.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
A. pratensis L.	.....	.....	.....	.....	.....	3	.....	3	.....	3	.....	3	.....	3	.....	1

[a] Numbers in table refer to the number of accessions tested of each grass species.

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
Arrhenatherum elatius (L.) Mert. and Koch.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
Avena sativa L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
Beckmannia erucaeformis (L.) Host.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
B. syzigachne (Steud.) Fernald	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
Bromus arvensis L.	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	.....
B. brizaeformis Fisch. and Mey.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. carinatus Hook. and Arn.	.....	.....	.....	.....	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2
B. catharticus Vahl	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3	.....	.....
B. commutatus Schrad.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. inermis Leyss.	.....	6	.....	6	.....	4	.....	6	.....	6	.....	6	.....	6	.....	1
B. japonicus Thunb.	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	.....
B. macrostachys L.	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	.....
B. macrostachys var. *lanuginosus* Boiss.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. marginatus Nees	.....	7	.....	7	.....	8	.....	4	.....	8	.....	8	.....	8	.....	.....
B. mollis L.	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	.....
B. polyanthus Scribn.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. purgans L.	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	.....
B. rigidus Roth.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. rubens L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. secalinus var. *velutinus* (Schrad.) Koch	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. squarrosus L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. tectorum L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. tectorum var. *glabratus* Spenner	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
B. vulgaris (Hook.) Shear	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
Dactylis glomerata L.	.....	8	.....	8	.....	8	.....	8	.....	8	.....	8	.....	8	.....	8
Deschampsia caespitosa (L.) Beauv.	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2	.....	2
D. danthonioides (Trin.) Munro	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
Elymus canadensis L.	1	2	.....	6	4	1	.....	5	1	4	2	3	1	4	.....	2
E. canadensis var. *robustus* (Scribn. and Smith) Makenz. and Bush	.....	1	.....	1	1	.....	.....	.....	1	.....	1	.....	.....	.....	.....	.....
E. condensatus Presl	1	1	.....	4	4	.....	.....	4	4	.....	4	.....	3	.....	.....	1
E. dahuricus Turcz.	1	.....	.....	1	1	.....	.....	1	1	.....	1	.....	1	.....	.....	1
E. glaucus Buckl.	2	1	1	3	2	2	.....	3	1	2	.....	4	.....	4	.....	.....
E. junceus Fisch.	1	.....	.....	1	1	.....	.....	1	1	.....	1	.....	1	.....	.....	.....
E. sibiricus L.	1	.....	.....	1	1	.....	.....	1	1	.....	1	.....	1	.....	.....	.....
E. triticoides Buckl.	1	.....	.....	1	1	.....	.....	1	.....	.....	.....	1	.....	1	.....	1
E. villosus Muhl.	.....	1	.....	1	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....
E. virginicus L.	.....	4	.....	4	3	.....	.....	4	.....	4	1	2	2	1	.....	.....
E. virginicus var. *glabriflorus* (Vasey) Bush	.....	1	.....	1	1	.....	.....	1	.....	1	.....	1	.....	1	.....	.....
E. virginicus var. *intermedius* (Vasey) Bush	.....	1	.....	1	.....	1	.....	.....	.....	1	.....	1	.....	.....	.....	.....
Festuca arizonica Vasey	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. elatior L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. elatior var. *arundinacea* (Schrib.) Wimm.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. gigantea (L.) Vill.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. idahoensis Elmer	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
F. obtusa Spreng.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. occidentalis Hook.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. octoflora Walt.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. ovina L.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
F. rubra L.	.....	1	.....	1	.....	2	.....	2	.....	2	.....	2	.....	2	.....	.....
F. rubra var. *commutata* Gaud.	.....	1	.....	1	.....	1	.....	.....	.....	1	.....	1	.....	1	.....	.....
F. thurberi Vasey	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
Holcus lanatus L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
Hordeum bulbosum L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
H. gussoneanum Parl.	.....	1	.....	1	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....
H. jubatum L.	.....	1	.....	1	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....
H. jubatum var. *caespitosum* (Scribn.) Hitchc.	.....	1	.....	1	.....	1	.....	.....	.....	1	.....	.....	.....	.....	.....	.....
H. murinum L.	.....	1	.....	1	.....	1	.....	.....	.....	1	.....	.....	.....	.....	.....	.....
H. nodosum L.	.....	1	.....	1	.....	.....	.....	1	.....	1	.....	1	.....	.....	.....	.....
H. vulgare L.	.....	2	13	2	.....	2	7	.....	.....	2	.....	2	.....	2	.....	2
Hystrix patula Moench	.....	.....	.....	.....	1	.....	.....	1	1	.....	.....	1	.....	1	.....	1
Koeleria cristata (L.) Pers.	.....	5	.....	5	.....	5	.....	5	.....	6	.....	6	.....	6	.....	6
Lolium multiflorum Lam.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
L. perenne L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
Millium effusum L.	.....	1	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
Phalaris arundinacea L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
Phleum pratense L.	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3
Poa ampla Merr.	.....	7	.....	7	.....	31	.....	30	.....	31	.....	31	.....	31	.....	7
P. arachnifera Torr.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
P. arctica R. Br.	.....	1	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. arida Vasey	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
P. bulbosa L.	.....	1	.....	1	.....	4	.....	4	.....	4	.....	4	.....	4	.....	1
P. canbyi (Scribn.) Piper	.....	3	.....	3	.....	7	.....	7	.....	7	.....	8	.....	7	.....	3
P. compressa L.	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3
P. curta Rydb.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
P. cusickii Vasey	.....	1	.....	1	.....	2	.....	2	.....	2	.....	2	.....	2	.....	1
P. cpilis Scribn.	.....	1	.....	1	.....	2	.....	2	.....	2	.....	2	.....	2	.....	1
P. gracillima Vasey	.....	2	.....	1	.....	3	.....	3	.....	3	.....	3	.....	3	.....	3
P. interior Rydb.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
P. juncifolia Scribn.	.....	1	.....	2	.....	4	.....	4	.....	4	.....	4	.....	4	.....	.....
P. nemoralis L.	.....	3	.....	3	.....	8	.....	8	.....	8	.....	8	.....	7	.....	1

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
P. nervosa (Hook.) Vasey	.....	.....	.....	.....	.....	2	.....	2	.....	2	.....	2	.....	2	.....	.....
P. nevadensis Vasey	.....	3	.....	3	.....	6	.....	6	.....	6	.....	6	.....	6	.....	3
P. palustris L.	.....	3	.....	3	.....	6	.....	6	.....	6	.....	6	.....	6	.....	3
P. pratensis L.	.....	14	.....	14	.....	15	.....	15	.....	15	.....	15	.....	15	.....	11
P. scabrella (Thurb.) Benth.	.....	1	.....	1	.....	3	.....	3	.....	3	.....	3	.....	3	.....	1
P. secunda Presl	.....	3	.....	3	.....	6	.....	6	.....	6	.....	6	.....	6	.....	3
P. sphondyloides Trin.	.....	2	.....	3	.....	5	.....	5	.....	5	.....	5	.....	5	.....	3
Polypogon monspeliensis (L.) Desf.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
Puccinellia distans (L.) Parl.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1
Secale cereale L.	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	.....	1	1	.....
Sitanion hystrix (Nutt.) J. G. Smith	1	.....	.....	1	1	.....	.....	1	1	.....	.....	1	1	.....	.....	1
S. jubatum J. G. Smith	1	.....	.....	1	1	.....	.....	1	1	.....	.....	.....	.....	1	.....	1
Trisetum flavescens (L.) Beauv.	.....	.....	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
T. spicatum (L.) Richt.	.....	1	.....	.....	.....	1	.....	1	.....	1	.....	1	.....	1	.....	.....
Triticum aestivum L.	1	1	.....	1	.....	1	.....	1	2	.....	.....	1	.....	1	.....	1

all the wild grasses were highly resistant. Some of the grasses tested by Mains were represented also in the present study, and all were highly resistant to culture 2. Thus, the results of culture 2 show that where a comparison is possible the results are comparable with those of Mains (10). Furthermore, with the exception of *Aegilops,* if those grasses susceptible to culture 2 had not been tested, the results would be, in fact, a confirmation of previous conclusions about narrow host range. Of course, it may be that the wheat mildew tested by the writer was different from that tested by all previous workers. However, since culture 2 did infect species of *Aegilops, Agropyron, Elymus,* and *Sitanion,* it is necessary to make adjustments of the previous concept of the pathogenic potentialities of wheat mildew. It also adds emphasis to the necessity of testing a large number of accessions of many grass species, if a better knowledge of pathogenicity is to be obtained.

Culture 3. On *Hordeum vulgare,* from Ann Arbor, Mich.

Culture 3 did not produce unusual results on barley varieties. Reactions of barley varieties were as follows: highly resistant—Arlington C.I. 702, Hanna C.I. 906; very resistant—Duplex C.I. 2433, Goldfoil C.I. 928, Heil's Hanna C.I. 682, 244 C.I. 1021; moderately resistant—Black Hulless C.I. 666, Common Chile C.I. 663, Lynch C.I. 919, Nepal C.I. 595; moderately susceptible—Coast C.I. 276, Oderbrucker C.I. 940, Peruvian C.I. 335; very susceptible—Horsford C.I. 877, and Malting C.I. 1129.

For the first time infection by barley mildew is reported on grass species outside the genus *Hordeum.* To culture 3 there developed a moderately susceptible reaction on *Agropyron intermedium* and *A. trachycaulum,* a moderately resistant reaction on *A. striatum,* and a very resistant reaction on *A. spicatum* and *Elymus glaucus.* These results, which record infection of species of *Agropyron* and *Elymus* by barley mildew, are in disagreement with all previous published records. Marchal (12) obtained no infection on any wild grass species except *Hordeum,* but his results included only one collection for each of the following species: *Agropyron caninum, A. giganteum, A. repens* and *Elymus arenarius.* If the few wild grass species that proved susceptible had not been tested, the remaining negative results would have confirmed the concept of previous workers that the specialized races of *Erysiphe graminis* were restricted in infection range to the species of a single genus. Apparently, insufficient collections of grass species were tested in previous studies.

The results obtained with culture 2 from wheat and culture 3 from barley show that these mildew cultures can infect wild grasses. Since some of the infected grasses exhibited susceptible and very susceptible reactions in the case of wheat mildew and moderately susceptible reactions in the case of barley mildew, the results suggest that these mildews might be discovered eventually on wild-grass species. The mixed cultures studied from *Agropyron repens* and *Elymus dahuricus* present such situations.

Mixed culture on *Agropyron repens,* from Yakima, Wash.

The inoculation results proved interesting, since in addition to infection of many grass species, 2 varieties of barley (Horsford C.I. 147 and Malting C.I. 326) showed a very susceptible reaction after inoculation with conidia from the bulk culture on *Agropyron repens.* The infection of these barley varieties by conidia from *A. repens* was verified in 3 additional tests under conditions that excluded the possibility of foreign contamination by employing the glass lamp-chimney technique. The mildew on barley was increased and designated culture 7. As will be explained in greater detail, culture 7 had a narrow host range on wild grasses and, besides barley, infected chiefly *Agropyron repens,* the original host. The other portion of the mixture, designated culture 6, had a wide host range, and many wild-grass accessions were available by which it could be separated from culture 7.

Culture 6. On *Agropyron repens,* from Yakima, Wash.

Culture 6, an *Agropyron* mildew isolated from a mixed culture on *A. repens,* as described above, proved to be the most aggressive mildew culture studied, both in number of grass collections infected and in severity of the attack. All collections of all species of *Agropyron* exhibited some degree of susceptibility to this culture. Furthermore, infection was produced on *Aegilops cylindrica, Elymus canadensis, E. condensatus, E. dahuricus, E. glaucus, E. junceus, E. canadensis* var. *robustus, E. sibiricus, E. triticoides, E. virginicus* var. *glabriflorus, Hystrix patula, Sitanion hystrix* and *S. jubatum.* Such a wide infection range greatly conflicts with the concept that *Agropyron* mildew infects only species of *Agropyron,* which was established by Marchal (12) and has not been contested heretofore. Culture 6 is perhaps different from all the other *Agropyron* mildews studied by previous workers. Marchal's studies contain only a few species of *Agropyron* and no species of *Aegilops, Elymus, Hystrix* or *Sitanion.* Since the work of Marchal (12) and Salmon (27) represents the only information available on *Erysiphe graminis* from *Agropyron,* it appears that the long-held ideas about pathogenicity were probably based on insufficient data.

Culture 7. On *Hordeum vulgare* by infection with conidia from *Agropyron repens* from Yakima, Wash.

This culture originated on *Agropyron repens,* but was multiplied on barley varieties. Reactions of differential barley varieties were as follows: very susceptible—Arlington C.I. 702, Goldfoil C.I. 928, Horsford C.I. 147, Malting C.I. 326, Peruvian C.I. 925; moderately resistant—Black Hulless C.I. 666 and Nepal C.I. 595.

The only wild grass exhibiting any appreciable susceptibility to culture 7 was the accession of *Agropyron repens* from which it originated. If it were not established by these experiments that culture 7 had its origin on *Agropyron repens* there would be no hesitancy in referring it to *Erysiphe graminis hordei.* Since culture 3 from *Hordeum vulare* infected certain

accessions of grass species in the genera *Agropyron* and *Elymus,* it seems logical that "barley mildew" should occur on such wild grasses under natural conditions. It could be interpreted that culture 7 represents a barley mildew occurring on *Agropyron repens.* Further evidence in support of this contention is the observation that there was a light infection of *Erysiphe graminis* on cultivated barley plants in the field adjacent to the plants of *Agropyron repens* on which culture 7 was originally collected. Culture 7 produced very striking results on barley varieties. The very susceptible reaction on the variety Arlington C.I. 702 distinguishes culture 7 from all described races of barley mildew in the United States. This discovery of a new race of *Erysiphe graminis* occurring on *Agropyron repens,* but capable of infecting barley, increases considerably the significance of the results showing that barley mildew can infect wild grasses under both natural and artificial conditions.

Mixed culture on *Elymus dahuricus,* from Ann Arbor, Mich.

A vigorous culture of *Erysiphe graminis* on *Elymus dahuricus* was brought into the greenhouse from the writer's grass-pathology nursery at the University of Michigan Botanical Garden. An inoculation of grasses and cereals provided unusual results. Infection was produced on both wheat and barley, as well as many species of *Aegilops, Agropyron, Elymus, Hystrix, Hordeum,* and *Sitanion.*

These results demonstrated that either there was a mixture of mildew races on *Elymus dahuricus,* or that there existed a race of *Erysiphe graminis* on *Elymus dahuricus* that could infect species of all these genera. Two isolates of this bulk culture were studied.

Culture 10. On *Triticum aestivum,* from Ann Arbor, Mich.

Culture 10 originated from infection on wheat (Malakoff C.I. 4898) by conidia from the mixed culture on *Elymus dahuricus.* The infected wheat plants were isolated in a separate greenhouse. This culture was maintained by transfers of conidia to fresh wheat seedlings at 2-week intervals.

Inoculation of grasses and cereals with culture 10 resulted in the infection of many grass species in the genera *Aegilops, Agropyron, Elymus, Hystrix, Sitanion,* and *Triticum* (Table 1). Reactions of differential wheat varieties were as follows: very susceptible—Malakoff C.I. 4898; moderately resistant—Axminster C.I. 1839. The reaction on the latter variety was confined to the first leaf sheath. Thus, culture 2 from wheat and culture 10, which infects wheat but came from *Elymus dahuricus,* infect many of the same grass species; but culture 10 infected many more collections of grass species than did culture 2.

Culture 11. On *Elymus dahuricus,* from Ann Arbor, Mich.

Culture 11 was isolated on *Agropyron desertorum,* which had been infected with conidia from the bulk culture on *Elymus dahuricus.* Only a

moderately resistant reaction on *Agropyron desertorum* resulted from inoculation with this culture. However, a few conidia were transferred to *Elymus dahuricus*, and the resulting infection provided a more vigorous culture with abundant sporulation for inoculation purposes.

From the results in table 1 it is seen that culture 11, an isolate from *Elymus dahuricus*, was capable of infecting only species of *Agropyron* and *Elymus*. *Agroypron* species were found to be more favorable for the development of culture 11 than were species of *Elymus*, both in number of species and degree of susceptibility. More species of *Agropyron*, however, were tested than of *Elymus*.

Culture 13. On *Elymus condensatus*, from Pullman, Wash.

This culture was started from dry leaf material sent by George W. Fischer, who obtained the inoculum from this grass growing in the Soil Conservation Service nursery at Pullman, Wash. Results of inoculations of grasses and cereals with this culture, multiplied on *Elymus condensatus*, are given in table 1. Culture 13 produced some degree of infection on nearly

TABLE 2.—*Summary of the reactions of 23 accessions of cereals and other grasses to 8 cultures of Erysiphe graminis*

Grass accessions	Cultures of *Erysiphe graminis* and source genus							
	Hordeum	*Triticum*	*Agropyron*	*Agropyron*	*Secale*	*Elymus*	*Elymus*	*Elymus*
	3	2	6	7	18	10	11	13
	Types of reaction							
Aegilops cylindrica 219[a]	0[b]	3–4	3	.....[c]	.....	0	0	0
Agropyron cristatum 153	0	0	2+	0	0	2+	0	2–
Agropyron desertorum 151	0	0	3–4	0	0	3–	2–	0
Agropyron inerme 108	0	0	2+	0	0	3	4	3
Agropyron repens 150	0	0	3–4	0	0	2–	3–4	0
Agropyron repens 370	.....	.....	4	3	.....	.....	.....	.....
Agropyron sibiricum 142	0	0	2–3	0	0	2–	0	1+
Agropyron smithii 152	0	0	2–3	0	0	0	3–	2–
Agropyron spicatum 130	2–	3	4	0	0	3	4	2+
Agropyron trachycaulum 121	0	0	2–3	0	.....	0	1	0
Agropyron trachycaulum 134	3	0	2–3	0	0	3	2–	3–
Elymus canadensis 162	0	3	1–2	0	.....	3–	2+	2+
Elymus condensatus 164	0	0	2–3	0	0	3–	2–	3–
Elymus dahuricus 176	0	3	4	0	0	3	4	2–
Elymus glaucus 173	0	2–	0	0	.....	1+	0	0
Elymus junceus 168	0	4	4	0	.....	4	2–	2–
Elymus triticoides 174	0	3	3	0	0	0	0	0
Elymus virginicus 214	0	0	2–3	0	.....	0	2–	4
Hordeum vulgare (Malting C.I. 1129)	4	0	0	4	0	0	0	0
Hystrix patula 252	.....	.....	3–4	0	0	2–3	0	.....
Secale cereale 371	0	0	0	0	4	0	0	0
Sitanion hystrix 179	0	3	4	0	0	2–	0	1–
Triticum aestivum (Malakoff C.I. 4898)	0	4	0	0	0	4	0	0

[a] Accession numbers given to grass collections by the writer.
[b] For explanation of these types of reaction see under Materials and Methods.
[c] A blank indicates no test was made.

all species of *Agropyron* and *Elymus* tested, and a very resistant reaction developed on *Sitanion hystrix*. Cultures 11 and 13 from species of *Elymus* are quite comparable in several respects. They infect species of *Agropyron* and *Elymus,* and the conidia of the two cultures are somewhat smaller than those of other cultures infecting grasses in the tribe Hordeae. Cultures 11 and 13 show a similar brownish color of their mycelia not observed in the other cultures. They, however, can be easily distinguished by differences in reactions of several grass accessions (Table 2). These two *Elymus* mildews differ from each other, but are closely allied and distinct from all other cultures studied.

In table 2 are given significant reactions of certain grass accessions and cereal varieties to the 8 cultures.

DISCUSSION

The results of this investigation demonstrates that the infection range of no culture of *Erysiphe graminis* here reported is restricted to the species of one genus. *Erysiphe graminis* hitherto has been divided into specialized varieties, each considered as limited to species of a single grass genus. Only a few instances of infection of species in closely related genera have been noted. The infection of species of *Aegilops* with wheat mildew by Reed (18, 19) and Mains (10) was not stressed because of the close relationship of *Aegilops* to wheat. The infection of *Arrhenatherum elatius* with oat mildew by Marchal (12) was confirmed by Reed (19). Reed assigns no importance to this fact because *Arrhenatherum* is taxonomically closely allied to *Avena.* The other and even more extraordinary example is the infection of *Hordeum silvaticum* (*Elymus europaeus*) with wheat mildew reported by Salmon (28). On the basis of the results of the present study the importance of the few records of infection of species of more than one genus by a given culture of *Erysiphe graminis* as noted by previous investigators appears to have been dismissed too lightly.

The 8 cultures of *Erysiphe graminis* described in this study have been shown by infection experiments to be pathogenically distinct. The differentiations were possible on the basis of the type of reaction produced on a group of grasses and cereals.

On the basis of the old concept holding that specialized varieties of *Erysiphe graminis* were restricted to species of a single genus, one would be justified in concluding, for example, that all races of *E. graminis* on species of *Agropyron* would be *Erysiphe graminis agropyri.* The results of the present study demonstrate that this is not necessarily the case. It might be *Erysiphe graminis tritici, E. g. hordei, E. g. agropyri, E. g. elymi,* or a mixture of these "specialized varieties." All of the above named "varieties" also could occur separately or as a mixture on certain species of *Elymus.*

The possibility of hybridization between different pathogenic races during the initiation of the perithecial stage is presented, since races that

attack *Triticum, Hordeum, Agropyron* and *Elymus* can occur together on the same plant of certain species of *Agropyron* and *Elymus.* Furthermore, because wheat and barley mildews are shown to infect grass species, wild grasses must now be considered in the epiphytology of the disease on cereals as sources of primary infection and perennial stations for the fungus. These statements are given considerable emphasis by the fact that a culture that could be classed as a new race of *Erysiphe graminis hordei* was isolated from *Agropyron repens* collected at Yakima, Wash., which produced a very susceptible type of infection on the barley variety, Arlington C.I. 702. This barley variety had been resistant to all 7 races of *Erysiphe graminis hordei* described for the United States by Mains and Dietz (11) and Tidd (30).

None of the cultures reported in this study can fulfill the requirement that a given specialized variety infects species of only one genus. However, if the descriptions of the various specialized varieties were emended to include the new infection results, most of the cultures could be assigned to previously recognized varietal names. Thus, culture 3 would be referred to *Erysiphe graminis hordei,* culture 2 to *tritici,* culture 6 to *agropyri,* and culture 18 to *secalis.* What, then, about the nomenclature of culture 10, which is comparable to "wheat" mildew but was isolated from *Elymus dahuricus* and culture 7 which is comparable to "barley" mildew but isolated from *Agropyron repens?* Do these cultures represent "wheat" and "barley" mildews on *Elymus* and *Agropyron,* or *Elymus* and *Agropyron* mildews infecting wheat and barley, respectively, or are they races not to be identified specifically as either gross or cereal mildew "varieties"? Cultures 11 and 13 might be considered as two races of *Erysiphe graminis elymi,* which was proposed as a morphologic variety by Homma (3) based on slight differences in conidial measurements.

This type of classification would still tend to obscure the pertinent points of new information, namely, that none of these cultures is restricted to species of a single genus, that several cultures overlap in host ranges, and that it is possible for 2 or more races, comparable to varieties of previous workers, to occur together as a mixture on certain wild-grass host plants, thus presenting the opportunity for hybridization between races. The use of varietal names may continue to be useful despite the difficulties that accompany their application in the instances described. It does not seem advisable to attempt a reclassification of the varietal system until more information is available.

Wide differences occur between the host ranges of the various cultures. Culture 6 from *Agropyron repens* infected the largest number of grass collections and species, including at least some degree of infection on all collections of all species of *Agropyron.* The rest of the cultures provide a gradating series of degrees of specialization down to culture 18 from *Secale cereale,* which besides this species produced a very weak infection on *Agropyron spicatum.*

All cultures reported here were collected from grasses in the tribe Hordeae and produced infection only on species in this tribe.

This study also has demonstrated that different strains within grass species vary considerably in reaction to *Erysiphe graminis*. These differences are of importance in the selection of economic grasses for disease resistance. Some grasses were outstanding for their resistance, the most noteworthy being *Agropyron trachycaulum* (183, 184, 185),[4] *Elymus virginicus* var. *intermedius* (208), *E. canadensis* (163), *E. glaucus* (172), *Hordeum nodosum* (177), *Lolium multiflorum* (158) and *L. perenne* (157). It must be remembered that these two species of *Lolium* might be susceptible to a mildew race from *Lolium* or from other grasses as yet not studied, and the other "resistant" grasses may be susceptible to other races of *Erysiphe graminis*.

In the studies of powdery mildew of wheat and barley, genetically uniform agronomic varieties have been available as differentials to distinguish the different pathogenic races. Few genetically selected strains of wild grasses are available at present, and most grass-disease investigations must employ variable collections of grass species. This is exemplified by the great variation of reaction to powdery mildew of plants within certain grass collections used in this study.

SUMMARY

Three hundred and eighteen accessions of 108 species and 9 varieties of wild and cultivated grasses of the genera *Aegilops, Agropyron, Agrostis, Alopecurus, Arrhenatherum, Avena, Beckmannia, Bromus, Dactylis, Deschampsia, Elymus, Festuca, Holcus, Hordeum, Hystrix, Koeleria, Lolium, Millium, Phalaris, Phleum, Poa, Polypogon, Puccinellia, Sitanion,* and *Trisetum,* as well as 15 varieties of *Hordeum vulgare,* 1 of *Secale cereale,* 2 of *Triticum aestivum* and 1 of *Avena sativa* were studied in regard to their reaction to 8 cultures of *Erysiphe graminis.*

In contrast to the previous concept of restriction of infection to a single host genus all the cultures studied produced infection on species of two or more genera.

Powdery mildew from wheat and barley infected wild grass species. Cultures comparable to wheat and barley mildews were isolated from naturally infected plants of *Elymus dahuricus.*

A virulent culture isolated from *Agropyron repens* infected the previously resistant barley variety, Arlington C.I. 702 and could be classified as a new race of *Erysiphe graminis hordei.*

Differences in reaction of strains within grass species were demonstrated in nearly every instance where infection was produced on a grass species containing more than one accession, and in a few instances differences in reactions of individual plants were found within accessions, thus affording an opportunity for selection for resistance. Several accessions of economic grasses have shown notable resistance to all of the cultures.

[4] Accession numbers given to grass collections by the writer.

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HOST–PARASITE RELATIONSHIP BETWEEN THE OAT PLANT (AVENA SPP.) AND CROWN RUST (PUCCINIA CORONATA)

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INTRODUCTION

Since 1705, when Tournefort (34), before the Académie Royale des Sciences, described parasitic molds as the cause of plant diseases and discriminated between those molds that obtain nourishment from living entities and those that thrive by inducing decay of inanimate materials, the problem of the relationship between host and parasite has been the object of no little study and speculation. We shall briefly recount the more important efforts to resolve that problem, a subject that can now be considered as a whole in the light of convergent cytological and cytochemical techniques.

REVIEW OF LITERATURE

In 1886, de Bary (6) published his classical paper on the parasitic behavior of *Sclerotinia libertiana* Fckl. He proved to his own satisfaction, at least, the presence of an active entity in the extracts of parasitized tissues of certain host plants subjected experimentally to the fungus. This entity or principle he found capable of inducing the disease effects he described. When he boiled the extracts containing this principle he succeeded in destroying its potency; whence his conclusion that the effect produced by this agent on the host-cell walls under its impact was none other than that of an enzyme.

We next find de Bary's deductions essentially confirmed by Marshall Ward (35), who, in a notable paper read before the Royal Society in London, concluded by way of summary that "all the evidence points to the existence, in the cells of the fungus, of enzymes or toxins, or both, and in the cells of the host-plant of antitoxins or similar substances, as the decisive factors in infection or immunity." He is frank to admit, however, that up to that time he had failed to isolate any such bodies.

Again, the facts as disclosed by de Bary were substantiated by Brown (9), who prepared from the germ tubes of *Botrytis cinerea* Auct. a markedly active extract. This he found capable of attacking and decomposing a variety of vegetable and fruit tissues and equally active in its effect on the leaves and floral organs of many plants, and the stems and leaves of numerous succulents. Heating at 60–70° C. effectively destroyed the extract. The author failed to separate the enzymic from the toxic principle, though he tried in various ways to do so.

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Stakman (29), after comparing the sequence of events in the infection of two varieties of *Triticum,* the one (Minn. 163) susceptible, the other (Khapli) resistant to *Puccinia graminis tritici* Eriks. and Henn., concluded that resistance to the rust is due to physiological incompatibility between host and parasite, a question calling for an answer through biochemical investigation. Later, the same author (30) states that the hypersensitiveness of the host seems to be common to plants that are somewhat resistant to *P. graminis tritici* and to those that are nearly immune from this rust. This hypersensitiveness becomes manifest very soon after fungal invasion of the host cells, and in some instances is visible even before such invasion occurs.

Reed and Crabill (25) observed in their study of cedar rust of the apple that an occasional rust-infection court died out at the center. They were of the opinion that this condition could be explained on the assumption that a deleterious product of the fungus diffused into cells adjacent to the invading hyphae and killed them before the fungus actually reached them. The rust organism, being an obligate parasite, is unable to grow in these dead cells; accordingly, its further progress is stopped. Thus, a seemingly paradoxical situation arises here. That is to say, the hypersusceptibility of the host is responsible for the death of the parasite.

In her study of infection phenomena resulting from invasion of Kanred wheat by a weakly virulent physiologic race of *Puccinia graminis tritici,* Allen (2, 3) observed that the cytoplasmic envelope of a growing haustorium of the invading mycelium disappears rapidly. This she interprets as a possible yielding by the fungus of some substance into the host cell. She observed that this contact between host and parasite was accompanied by a chemical change, but was unable to determine whether such change was due to the "mere physical presence of the haustorium or to some substance diffusing from it." Any attempt to disclose the basic facts underlying the true nature of rust resistance by commonly accepted cytologic technique cannot prove otherwise than unsatisfactory. It is too easy to mistake mere artifact for the actual and characteristic. Any interpretation of what one may, therefore, observe as having taken place in fixed and stained infected or other tissue as compared with the intravitally stained tissue cannot be other than an approximation of the truth.

Rice (27), in her studies on the nature of rust parasitism, concludes that the intrusion of the haustorium induces "offensive action" by the host nucleus. Again, she refers to Allen's (1) description of the relationship of the fungus to its resistant host as a "host-nuclear offensive," which, as she interprets it, is indicative of a chemotactic response to substances that diffuse out into the host cell from the haustorium. The reaction manifested by the invading fungus she interprets as induced by secretion from the host cells. Sappin-Trouffy (28) is of the opinion that through contact of the fungus haustorium and the host nucleus the invading parasite takes up certain products of host-cell metabolism. As to what these products are or how they become available to the parasite he does not say.

Pady (24), in his consideration of infection in *Gymnoconia interstitialis* (Schl.) Lagh., calls attention to the amount of mycelium within the leaf tissues of *Rubus* prior to formation of haustoria, and infers that some nourishment must derive directly to the fungus from the host cells with which the hyphae of the invading organism are in intimate contact. Thus does he add the weight of his opinion to that of other students of host-parasite relationship; but we are, up to this point, busy with spade work necessary to the disclosure of the means and *modus operandi* necessary to the establishment and maintenance of such relationship.

Ward (36) was the first to demonstrate experimentally the dependence of plant rusts on carbohydrates or on certain intermediate products of photosynthesis elaborated by their hosts. He proved, for example, that the mycelium of *Puccinia glumarum* (Schm.) Eriks. and Henn. would succumb to starvation in wheat leaves kept in an atmosphere void of carbon dioxide.

Mains (22) demonstrated experimentally the relation of light and carbohydrates to cell respiration of certain rust-infected plants. In his investigation of the carbohydrate relationship he found that in the absence of either light or carbon dioxide the growth of *Puccinia coronata* Corda or *P. sorghi* Schw. was retarded or stopped altogether because of dearth of carbohydrates. When he supplied these in the form of sugar solutions, *P. sorghi* resumed growth, even in the dark, on corn seedlings deprived of their endosperm.

A year later Dufrenoy (14a and b) published his observation on the accelerated transpiration and fatty degeneration of tissues affected by rust.

Arthur *et al.* (4) incline to the opinion that the rust fungus gains access to the host cell through a minute pore in the cell wall effected through an enzyme brought to bear on the cell wall at a point of contact between the haustorium mother cell and the host cell. They refer, too, to the "delicate balance" that apparently is established between the rust organism, on the one hand, and the host cells of a susceptible variety or species, on the other, with a consequent increase in size of the host nuclei of attacked cells and an accelerated metabolism in such cells. Rust-resistant plants show no such balanced behavior. Biochemically considered, as we shall presently see, they cannot.

Certain contributors to our knowledge of rust-infection phenomena, notably Beauverie (7) and, more recently, Thatcher (31, 32), have emphasized the evident influence of an active parasite on the "antibiotic struggle" that characterizes the host-parasite relationship.

Beauverie (7) is convinced that the destructive action of the parasite brings about a perturbed osmosis. The fungus, having penetrated the host tissue, takes up water, and the more so when its fructification is externally apparent, a stage of fungal development marked by a heightened respiration. All this results in a concentration of the cell sap and a dehydration of the cytoplasm. He states further that the fungus may exert an indirect

action, *i.e.*, secrete some diastases, under whose influence such large-molecule substances as starch, albumins, etc., are split into smaller molecules, more numerous, and crystalloid. There follows a marked increase in osmotic pressure.

Thatcher (32) demonstrated that in the germ tubes and haustoria of *Uromyces fabae* (Pers.) de Bary on *Pisum sativum, U. caryophyllinus* (Schrank) Wint. on *Dianthus caryophyllus, Botrytis,* and *Sclerotinia sclerotiorum* (Lib.) Mass., the osmotic pressure registered by the fungus was greater than that of the host, thus confirming MacDougal's (16) observation of the same phenomenon in his study of induced parasitism. Thatcher proved by means of permeability measurements of the tissue of rust-infected and noninfected plants that rust infection is attended by increased permeability of the plasmatic membrane of the host cells. This increased permeability he is inclined to attribute to some secretion from the rust fungus. On this observation Thatcher has based his hypothesis that "even an obligate parasite causes certain substances to leach from the host cells in its vicinity." It is his opinion that this hypothesis permits the extended survival of parasitized cells commonly observed among plant rusts. He suggests further (32) that the greater osmotic pressure of the parasite enables it to acquire water from contiguous host cells and that increased permeability makes available to the parasite those cell solutes to which the plasma membrane is no longer semipermeable. The author states further that this change in permeability is not the only physiological modification of the host cell essential to rust nutrition. He does not, however, suggest that dextrose is the source of the carbon utilized by the rust fungi.

More recently, Thatcher (33) states that his observation of living sections of rust-infected tissue over a period of several years has convinced him that extracellular rust enzymes may have a pertinent relationship to such differences in host response as characterize the mesothetic reaction. In several instances he observed extracellular fatty materials, which he interpreted as a sequel to rust invasion. Also, from time to time he had noted evidence of changes in the hydrophily of the host protoplasm. These several observations, "considered in relation to the universal secretion of enzymes by parasitic fungi and to the supposed nature of the plasmatic membrane," led him to conclude that there probably is some truth in the suggestion that the "rust fungus secretes at least two enzymes," one a protease and the other an enzyme capable of splitting lecithins from lecithoproteins, in short a "lipase."

MATERIALS AND METHODS

The investigation here considered was confined to crown rust (*Puccinia coronata*), cultured in field and greenhouse at Louisiana State University in 1942 and 1943. Inoculations in the greenhouse were made with urediospores of physiologic race 1, obtained from Beltsville, Maryland. Because

of their several types of specific response to infection by this physiologic race, young plants of the following oat varieties were inoculated on November 4, 1942, under nearly identical conditions: Bond, Victoria, Rainbow, Markton, Richland, and Bond × D69. Similarly, seedling plants of these same varieties were inoculated in December, 1942, and in January and February, 1943.

Tangential freehand sections of host tissue from immediately beneath the rust sorus were immersed in saline or sucrose solutions containing 0.1 per cent neutral red for vital staining. Other and similar sections were immersed in a saturated solution of 2–6 dichloroquinone imide in a 0.2 per cent solution of sodium barbiturate buffered at pH 8.6. Still other sections were immersed in a 0.01 per cent solution of para-phenylenediamine chlorohydrate, and some in a molybdenum reagent (H_2SO_4 20.8 cc., diluted by adding distilled water to 250 cc.) in which was dissolved 6.41 g. of ammonium molybdate. To 5 cc. of this was added, at time of application, 1 cc. of the following: 5.75 g. of sodium bisulphite in 85 cc. of distilled water + 0.5 g. 1-amino-2-napthol-4-sulfonic acid + 5 cc. of a 20 per cent solution of sodium sulphite.

The convergent use of these techniques gave a good picture of the comparative behavior of the phenolic compounds, of the polyphenol oxidases (as evidenced in decompensated respiration), and of the distribution and relative dispersal of the phosphorus compounds in the resistant or susceptible varieties of oats.

Of the reagents employed, the molybdenum proved most valuable, as the blue phospho-molybdenum compound forms almost instantaneously where phosphorus is free and more slowly where the phosphorus is more firmly bound into nucleoproteins, phosphorylated sugars, and lipoproteins. Thus, by means of the microscope, a proper idea of the dispersal of such compounds in rusted tissues was obtainable from sections of host material that had been immersed for stated periods of time ranging from a few seconds to several hours.

This same reagent also affords a picture of the oxidizable polyphenols in the tissues—one that conforms to the pictures obtained through the use of the para-phenylenediamine or the chloroimide reagents. Here, again, in all cases, the expression of the definitive coloration at the site of oxidizable phenols required from a few minutes to hours or even a few days. It was found advantageous, when sections had to be examined repeatedly for days, to preserve them in the refrigerator between periods of examination. The stock solutions of the reagents also were similarly stored.

CYTOLOGICAL CONSIDERATIONS BEARING ON THE VACUOLAR SYSTEM OF THE HOST-PLANT CELL

The response of the host-plant cell infected by a rust fungus may vary all the way from abrupt structural changes, resulting in sudden death, such as characterizes hypersensitivity flecks, to a progressive evolution of cell constituents, giving the host cell and its parasite the mutual benefit of a

long survival. When the leaf tissues contiguous to the infection court remain green, while the rest of the leaf becomes yellow, the infected cells even may enjoy a prolonged life.

Those two extremes of host response and all intermediate aspects can now be interpreted cytochemically in the light of recent knowledge of the interrelationship between the respiratory systems of the living cell and its cytological structure.

The ability of a parasite to thrive in the tissue of its host has been correlated (20, 21, 32) with its higher osmotic pressure: the 24 per cent solution of sucrose, which plasmolyzes the cells of the oat plant proximate to the court of infection, does not plasmolyze the hyphae of *Puccinia coronata* nor the host cells that lie beyond the court of infection.

This plasmolysis of affected cells, followed by deplasmolysis on prolonged immersion in the sucrose solution, has been interpreted as evidence of "increased permeability." But the vacuolar solution in the cells about the court of infection, where plasmolysis may be observed, shows other changes besides those ascribable to reduced osmotic pressure: 1. The vacuolar solution in these cells tends to become dispersed into numerous vacuoles differing not only in shape but also in the nature of their vacuolar solution. This dispersal of the vacuolar solution was recently considered by Reed and Dufrenoy (19) in that part of their paper devoted to cell secretion. 2. Coacervation, according to Dobry (13), intervenes as a limiting factor, so affecting the osmotic pressure as to render it almost nil. Coacervates of differing composition may coexist within the cell without any interfering osmotic phenomena. Through coacervation, cells may store substances in the liquid state where they remain available for metabolism, although they are as effectively protected, physicochemically, as though they were in a solid state. The vacuolar compounds that most directly pertain here are monophenols such as pyridoxin (vitamin B_6), diphenols (catechol), and oxidation-polymerization products. Normally, there is present in the vacuolar solution of the guard cells of the stomata and in that of the intervening epidermal cells of leaves of the oat varieties Victoria, Bond, etc., a compound that stains an intense blue with 2–6 dichloroquinone imide. In the other cells the compound may be too dilute to give a perceptible color reaction within the vacuolar solution, but becomes perceptible when coacervated.

Plasmolysis does not actually afford a way of measuring "osmotic pressure" in the vacuolar solution, as the osmotic pressure computed from the freezing point of expressed sap may be significantly lower than that calculated from plasmolysis. There exist within the living cell, besides the physical forces that play as osmotic pressure, some forces pumping water into the vacuole, from outside the cell, in the same way that there are forces active in the absorption of solutes. These forces involve an expenditure of energy. That energy is furnished by respiration—more precisely, by that specific part of respiration linked up with the cell structure and dependent upon the integrity of that structure. Commoner and Thimann (12) empha-

sized the interrelationship of the auxin action and cell respiration in the utilization of malic acid during growth of the coleoptile of oat seedlings, but present no data to account for the forces concerned in cell expansion. Berger and Avery (8), on the other hand, found in the coleoptile a number of the components of the Szent-Györgyi-Krebs respiration phosphorylation cycle, among which malic dehydrogenase was prominent.

The mechanism concerned in the transfer of energy from the oxidative to the assimilatory process appears to be a phosphate cycle with storage of the energy as phosphate-bond energy, and its release by subsequent splitting of the phosphate esters (23).

The phosphorus linkages operate in dehydrogenase systems whose activity is seemingly dependent on the cytological structure of the cell. The decline or "phospholyse" of the phosphorylated cytological constituents becomes evident in "fatty degeneration" of and the advent of myelin figures in the cells (Fig. 2, *ap*).

Following the course of one-half the glucose passing through this cycle, five "energy-rich phosphate bonds" may be derived, two on the fermentation pathway of the glucose passing from glucose to pyruvate; two as acetylphosphates, with oxidation of the two alpha-keto acids (pyruvic and keto glutaric); one with Kalckar's enol-phosphate, found on oxidation of fumaric or malic acid (15). Therefore, the transfer of one mole of triose through the cycle involves about one energy-rich phosphate bond for each of the six primary dehydrogenations.

Respiratory processes in the cell involve a series of phenomena: (a) phosphorylations; (b) oxidations or dehydrogenations of the phosphorylated metabolites; (c) synthesis of glucids from the products of the dehydrogenations, at the expense of the energy thereby liberated ("Pasteur reaction") (15).

Synthesis of glucids evidencing the Pasteur reaction may explain how concentration of sucrose may increase in parasitized tissues, or how starch becomes synthesized where it would not normally occur, or it may account for an increase in osmotic pressure.

From the time of Lavoisier the fundamental question as to how atmospheric oxygen, at relatively low temperature, acquires the property of performing the work of partial and complete oxidation has claimed the attention of both physiologists and chemists.

The experimental and theoretical study of catalytic auto-oxidation brought to the fore the "activation" of molecular oxygen, either (a) by addition to a nonsaturated system, or (b) by the activating influence of a metallic catalyst.

The first possibility involves oxidases or substances of nonsaturated character that absorb molecular oxygen and, in part, utilize it in the oxidation of other materials in the process of metabolism.

Simultaneously, hydrogen peroxide is produced by the hydrolysis of those primary oxides, and, by means of peroxidases, is activated for the purpose of oxidation.

Oxidases perform, not as catalysts, but as oxidizing enzymes, substances that undergo chemical change. For instance, we cannot dissociate "polyphenol oxidases" from the polyphenols while being oxidized (dehydrogenated) to quinoids.

The second possibility involves metals that can exist either as *ous* or *ic* compounds.

Iron, long ago (1705), was detected in the ash of plant tissues. Almost simultaneously it was found in chemical solution in plant tissues; but it took two centuries for iron to be recognized as the active group of cytochrome oxidases or of hydrogenases.

Hydrogenase is an iron compound active in the ferrous, but inactive in the ferric form, and behaving as the cytochrome-like "Pasteur enzyme" (17).

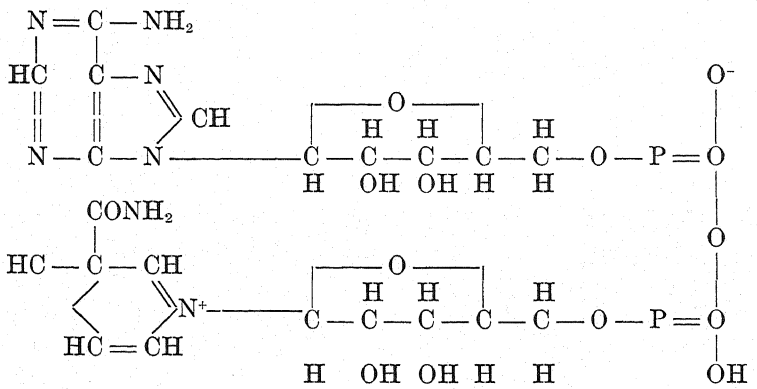
There are two ways of blocking the oxidations in the cell: either by replacing competitively the oxidizable metabolite by an unoxidizable compound (such as KCN), or by replacing O_2 by a nonoxidizing agent (such as CO).

As to hydrogenase activity, the importance of the phosphorus relationship is twofold, *i.e.*, in the phosphorylation of the coenzyme and in the phosphorylation of the hydrogen donor.

Dehydrogenases are dehydrogenating enzymes, activating hydrogen in phosphorylated glycerol or glyceric acid. The action of dehydrogenases is followed by that of the co-dehydrogenases, which transfer hydrogen. Following the discovery of these agents, it became clear that oxygen uptake is, to a marked degree, independent of CO_2 formation; hence, we can conceive of compensated *vs.* decompensated respiration, not in terms of O_2/CO_2 balance but in terms of reversible *vs.* irreversible dehydrogenations of metabolites.

The need of a coenzyme for the lactic, malic, and beta-hydroxybutyric dehydrogenases has been known since the systematic study of their properties.

Following is the structural formula for coenzyme I, diphosphopyridine nucleotide. (See Ann. Rev. Bioch. **10**: 230. 1941.)



Those dehydrogenases needing a coenzyme for their activation are inhibited by nucleotidases, which liberate inorganic phosphorus from the phosphorus linkages.

Examination of those substances that are oxidized in the cell discloses the fact that, in general, they are such as undergo the chemical changes involving loss of hydrogen (*e.g.,* in dehydrogenation) concomitantly with the building up or breaking down of energy-rich phosphorus linkage.

Phosphorus is the biochemical element *par excellence.* "Phosphorus mirabilis," as soon as it was discovered, attracted the attention of Huygens in France, of Boyle in England, of Leibnitz in Germany. Since then its biochemical prominence has become progressively evident, and in the study of our present problem we shall focus our discussion on phosphorus linkages.

Phosphorus Metabolism

The rust, as an obligate parasite, draws on the host cell for all of the materials it metabolizes after its reserves within the urediospore have been exhausted by the germ tube, appressorium, and stomatal vesicle. The cell contents of the urediospore was, prior to germination, rich in phosphorus that was strongly bound and capable of remobilization on germination of the spore. When this original supply of reserve material is exhausted and the phosphorus is again bound into energy-rich linkages within the germ tube, the appressorium, and stomatal vesicle, all of the phosphorus needed by the growing hyphae must be derived from the host.

The comparative cytochemical study of rust on a resistant and a susceptible host discloses a direct correlation between the ability of the rust fungus to produce an extensive mycelium and sporulate, on the one hand, and its ability, on the other hand, to draw upon a wider expanse of host tissue for phosphorus.

A normal cell may be assumed to be permeable to $PO_4^{\equiv}$ or to the salts of $PO_4^{\equiv}$ with cations, such as K^+, but impermeable to large molecules wherein PO_4 would be tied up by pentose phosphorylation to proteins, as in nucleotides. Tissues stained with the molybdenum reagent bring out the fact that the cells affected by rust and surviving infection release phosphorus compounds. In this respect they behave like cells that have been irradiated by ultra-violet radiations at sublethal doses (19) and that release phosphorus compounds acting as growth-promoting substances for neighboring cells. It may, therefore, be inferred that surviving infected cells release to the intercellular spaces phosphorus compounds that can be remetabolized by the rust hyphae, but that, also, inasmuch as they are not recaptured by the hyphae, may induce some reactions in the cells around the court of infection.

Therefore, gradients of phosphorus concentrations would become established not only between the infected host cells and the rust hyphae, but, as a whole, between the tissues of the court of infection and those immediately surrounding. There would result an influx of available phosphorus around

the actual court of infection into those tissues that face impending invasion by the encroaching hyphae, contrary to what would happen in healthy tissue.

Phosphorus is likely to be translocated there from the more remote tissues, mostly in the form of phosphorylated sugars. As the phosphorus thereby made available to the growing hyphae is being consumed the residue would be deposited in the form of glucose, sucrose, or starch; therefore accounting for that seemingly paradoxical result reported by Allen (1) in the case of mildew on wheat leaves, where high carbohydrate content and high respiration are intimately connected with growth of the parasite. That correlation would seem to be a general one and to be necessary in the case of all obligate parasites, rusts as well as mildews.

NONCOMPENSATED RESPIRATION

High respiration in infected tissues is concomitant with noncompensated respiration, and noncompensated respiration is concomitant with the disturbance of cell structure; noncompensated respiration means that O_2 absorption is increased without CO_2 emission being correspondingly increased, as part of the O_2 is used up for the dehydrogenation of the phenolic compounds to quinones, with formation of H_2O. The ultimate result of the interaction between rust and infected cells or between those infected cells within the infection court and those beyond that specific area will depend upon the severity of the decompensation of respiration, which, itself, will depend upon the dispersion of the dehydrogenases.

A disturbance of the energy-rich phosphorus linkages, as the cell faces infection, will result in a severe decompensation of respiration: phenolic compounds in the vacuolar solution, being no longer protected from irreversible dehydrogenation, form quinones, which diffuse out of the vacuolar solution into the cytoplasm, which they "fix" (26), and thus give rise to the hypersensitivity flecks (Fig. 1). The cells in those flecks are killed so quickly that they have no chance to induce metabolic responses in the nearby surviving cells.

Quite different is the case when noncompensated respiration allows the concomitant survival of infected cells and infecting hyphae. Here, such respiration is not made evident by the dehydrogenation of the phenolic compounds to brown derivatives and by the discoloration of the tissues, as in hypersensitivity flecks; but it can be demonstrated by immersing freehand sections of infected tissue in such reagents as para-phenylenediamine, 2.6 dichloroimide . . . , which give bright-colored quinoid derivatives on being dehydrogenated by the noncompensated activity of the oxidases.

Vital staining with those para or ortho compounds yielding bright-colored quinoid derivatives on dehydrogenation demonstrates that there is widespread in plant tissues an enzyme corresponding to what was known originally as tyrosinase. This enzyme catalyzes not only the aerobic oxidation of polyhydric phenols, where the $-OH$ groups are in either the para or ortho position, and the oxidation of the amines, where the $-NH$ groups

are in either the para or the ortho position, but, in the presence of catechol, also catalyzes the aerobic oxidation of certain monohydric phenols.

Whether tyrosinase, as here defined, represents a specific enzyme or a group of enzymes among which such specific enzymes as polyphenol oxidases or catechol oxidases should be discriminated, has been the subject of much recent controversy. What we are here concerned with in contributing to our understanding of the host-parasite relationship are these facts, *i.e.*, that the aerobic oxidation of various ortho-dehydroxy phenolic compounds is catalyzed at widely different rates by tyrosinase and that the oxidation

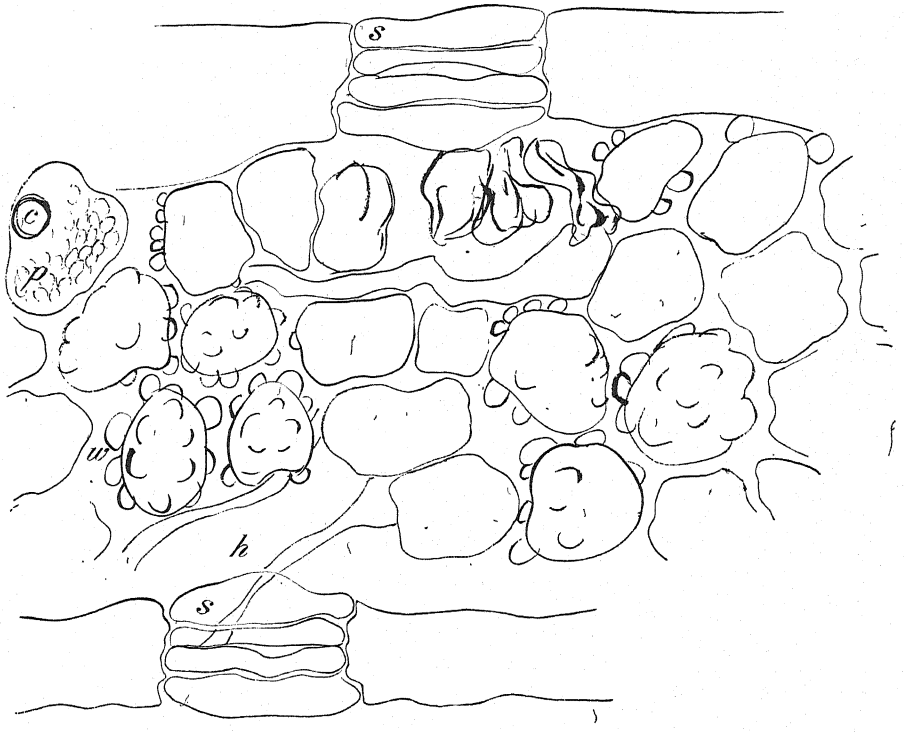


FIG. 1. Tangential section of rust-infected leaf of the variety Victoria. Inoculated November 4; examined November 10 in a solution of 2–6 dichloro-quinone imide. The penetration of the hyphae (*h*) through the stomata resulted in the collapse of the cells immediately adjoining, as evidenced by the folding in of the cell walls. The cells farther away show extrusions homologous to those featured by R. Allen as "pectic warts" (Jour. Agr. Res. [U.S.] 34: (8), pl. 3, fig. C, pp. 702–703. 1927).

products of these compounds vary in their tendency to inactivate the tyrosinase.

For instance, Baker and Nelson (5), in 1943, point out that catechol is oxidized very rapidly to quinones, as evidenced by the rapid browning of the reaction mixtures. They assume that these quinones exert an inhibitory effect on some intermediate link in the respiratory system of the living cells. Conversely, protocatechuic acid (4-carboxycatechol) is oxidized more slowly, yielding quinone at such a slow rate that the rest of the respiratory system

is able to reduce the quinone as soon as it is formed, thereby enabling the acid to serve as a hydrogen carrier. Baker and Nelson obtained their results from a study of the respiration of living cells of potato-tuber tissue. Slices of the tuber were subjected to a Warburg respirometer, which enabled them to record the amount of oxygen intake and CO_2 outgo, at a temperature of 25° C. Experimental evidence thus obtained supports their conclusion that at least 85 per cent of the oxygen uptake of such respiring tissue enters the chemistry of the potato cell by way of a tyrosinase-catalyzed oxidation.

From the foregoing investigations we may derive the following concept, which bears directly upon the general problem of host-parasite relationship, *i.e.,* that there exist in plant tissues, concomitantly, various phenolic substances that can be dehydrogenated by an enzyme system of the tyrosinase type. The dehydrogenation may be reversible, and those substances then act as hydrogen carriers as long as the respiration is not severely decompensated. Indeed, a slight decompensation, corresponding to some disturbance of the cytological structure, entailing increased permeability, may step up the dehydrogenation of monophenols to polyphenols and of polyphenols to quinones with correlative increase of O_2 intake that enhanced respiratory activity, which corresponds to a feverish condition of the tissues, is observed whenever cells survive attack by a given parasite; but they survive because they can keep up a dehydrogenase system whereby the quinones can be reverted back to the polyphenols; in other words, they will survive as long as they can retain a certain specific structural relationship between the phosphorus linkages and the cell constituents, as well as preserve the integrity of some – SH groups linked up with the nucleoproteins acting as dehydrogenases.

Wherever the quinones fail to be rehydrogenated, they will catalyze further oxidations, inhibit some links in the respiratory systems, and bring about death of the cells and formation of brown necrotic areas in the rust-infected leaf. The interrelationship between host and parasite as evidenced by resistance or susceptibility seems, therefore, largely dependent on the rate of dehydrogenation of polyphenols to quinones. Where this rate is not so rapid as to outstrip the rate of reversible reaction, the cell or cells can survive; both host and parasite can more or less compatibly live together; the host is susceptible. If the rate of quinone production is too rapid, the cells are killed before the rust fungus can get anything from them (Figs. 1 and 2). Thus no continuing parasitic relationship can be established, and the host is resistant. In this way decompensated respiration can be detected wherever hyphae of the rust grow and appropriate unto themselves phosphorus compounds from the host cells. A gradient of phosphorus concentration between the hyphae and the infected cells is demonstrated by staining with the molybdenum reagent. If the cells become too seriously depleted in phosphorus, they become the site of phenolic dehydrogenation to quinones, their cytoplasm becomes fixed by the quinones and they die. If, however,

they survive, they exhibit, concurrently with decompensated respiration, definite changes in structure, involving mostly the cytoplasmic interphases. These changes may be properly described as secretion phenomena: The central vacuole, originally present in the normal cell, may secrete a number of secondary vacuoles; the vacuolar contents, the colloids of which are chiefly

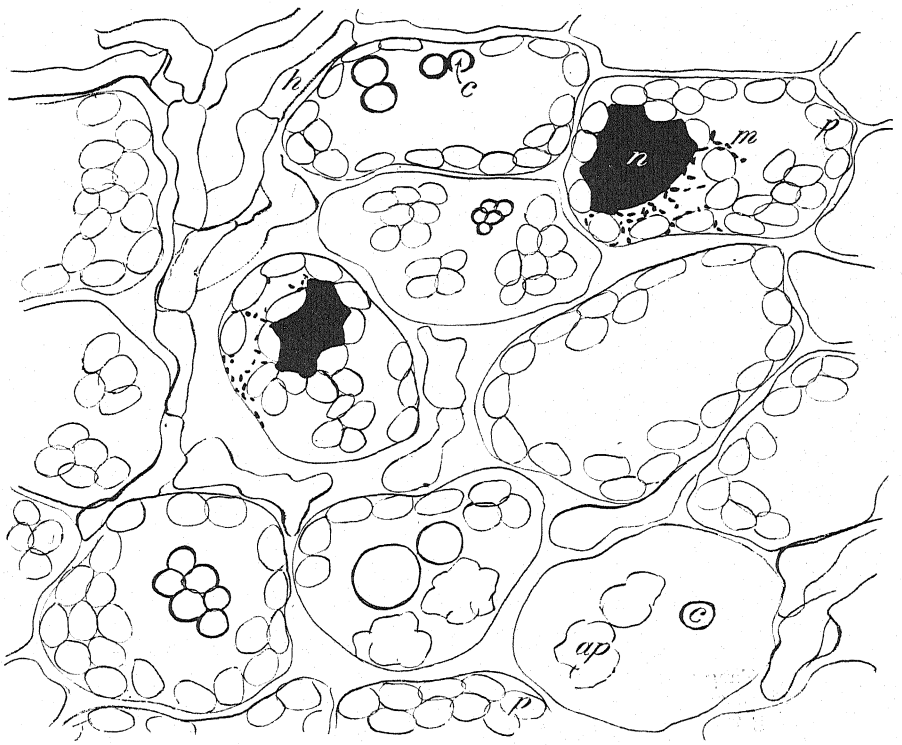


FIG. 2. Tangential section of rust-infected leaf of the variety Victoria below sorus of *Puccinia coronata*. Infected December 30, 1942; examined January 28, 1943; section treated with the molybdenum reagent. In two of the cells the nucleus (*n*) stained a deep blue, as well as neighboring mitochondria (*m*) (featured solid black). Intercellular hyphae (*h*) show no material staining blue with the molybdenum reagent, indicating that whatever phosphorus had been available has been translocated to the few urediospores formed (not shown). The chloroplasts (*p*) (merely outlined) remain appressed against the cell wall in some cells, and their distribution outlines the central vacuole, wherein the phenolic compounds have become coacervated into a number of spherical bodies, sharply outlined by the limiting membrane (*c*). In other cells the chloroplasts became agglutinated (*ap*), eventually losing their identity and fusing into a "myelin body."

represented by phenolic compounds, may not be apportioned equally to all of those vacuoles.

Cell Secretion

Normally, the volume of the oat-leaf cell is occupied for the most part by the vacuolar solution, rich in colloids, represented chiefly by phenolic compounds. That vacuolar solution stains a deep purple or violet with neutral red, occupies a vacuole in the central part of the cell, and is surrounded by the cytoplasm. The cytoplasmic vacuolar interphase is the site

of surface-tension phenomena, allowing for the irregular shape of the vacuole.

As the cell is affected by the parasite, the vacuolar solution becomes dispersed into several vacuoles. This dispersal may be considered as representing a phenomenon of external secretion (as contrasted with internal

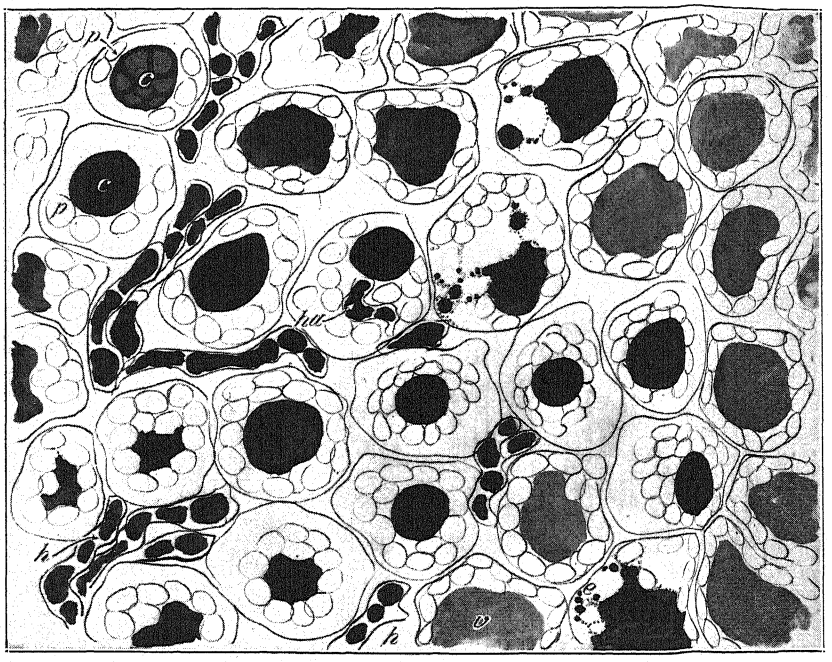


FIG. 3. Tangential section of rust-infected leaf of the variety Markton below sorus of *Puccinia coronata*. Inoculated December 30, 1942; examined February 17, 1943. Vital staining with neutral red in 24 per cent sucrose solution. Vacuolar material staining purple to orange, featured black or grey. The vacuoles in the intercellular hyphae (*h*) contain several small vacuolar precipitates, shown in solid black. In the vicinity of the hyphae the cells of the chlorenchyma show plasmolysis in the 24 per cent sucrose solution; the chloroplasts (seen in outline only) become agglomerated, as the cytoplasm retracts from the cell wall, around the contracting vacuole. Where the cell plasmolyzes, the vacuolar solution stains orange or red, as contrasted to pink or purple in the normal cells, indicating a shifting of the pH from the normal 5.5 towards 7. In many cells in the vicinity of the hyphae, the phenolic compounds, and particularly the pyridoxin from the vacuolar solution, became coacervated into a spherical mass (*c*), sharply outlined by deeply staining limiting boundary. As the vacuolar solution around the coacervate undergoes plasmolysis, the cytoplasm, carrying the chloroplasts, may become tightly appressed against the coacervate. In some cells, the vacuolar solution shows no coacervation; instead, it evidences an excretion or a secretion of materials to the outside, as the vacuole becomes irregularly lobed and buds out peripheral vacuoles sharply outlined by a row of lipid droplets in the enmeshing cytoplasmic strands. Farther away from the hyphae, the cells retain their turgor in the 24 per cent sucrose solution, the vacuolar solution (*v*), stains pink to purple, and occupies the huge central vacuole pressing the cytoplasm and plastids against the cell wall.

secretion, presented under the heading "coacervation") (Fig. 3). The original vacuole may form "buds," which become detached as so many secondary vacuoles. Conversely, these secondary vacuoles may lack in colloidal material. At the interphase, with interfacial surface tension, each vacu-

ole tends to become perfectly spherical. Neutral red stains the contents of these secondary vacuoles orange red, thus demonstrating a shift of pH from normal acidity (approx. pH 5.5) toward neutrality. These smaller spherical vacuoles are the site of highly noncompensated oxidase activity, and give a bright-color response for quinoids on treatment of the tissues with para-phenylenediamine or 2–6 dichloro-quinone imide.

Concomitant with that secretion of materials from the mother vacuole into the newly formed smaller peripheral vacuoles, in the same cell, there occurs a secretion of intracellular material into the intercellular spaces correlatively with what has been discussed as "increased permeability."

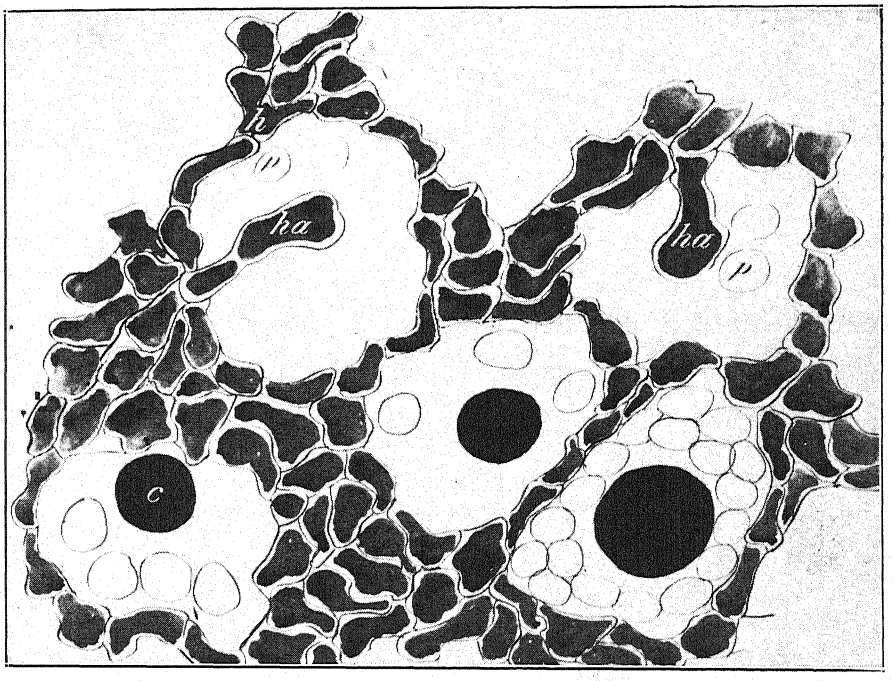


FIG. 4. Tangential section of rust-infected leaf of the variety Rainbow below sorus. Inoculated December 30; examined January 28 in a solution of neutral red in 24 per cent sucrose. The cells of the chlorenchyma are enmeshed in the pseudo-tissue of the intercellular hyphae (*h*), comprising cells each of which contains a large red-staining vacuole (featured grey). The two upper chlorenchyma cells contain each an haustorium (*ha*) filled with the vacuolar solution occupying one or two vacuoles. The lower cells contain each a coacervate (*c*) with a deeply staining limiting layer surrounding a lighter stained core (featured solid black), (*p*) chloroplasts, in outline.

Coacervation

The most conspicuous type of secretion occurs within the vacuolar solution itself through that particular process described by Bungenberg de Jong (10) as "coacervation." The colloidal materials, originally uniformly distributed throughout the vacuolar system, may become unequally apportioned to various drops therefrom, as the central vacuole secretes smaller vacuoles at its periphery, as described above.

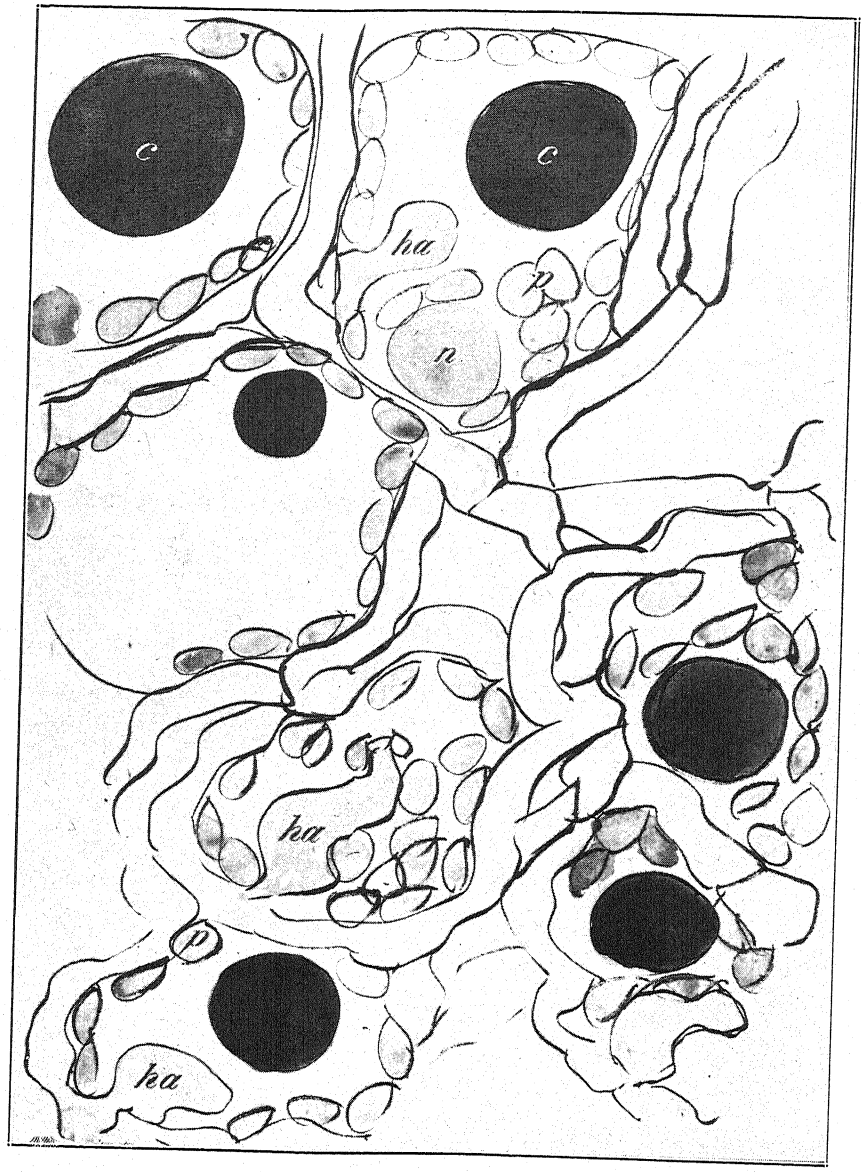


FIG. 5. Bond inoculated with crown rust from Bond January 14; examined February 14, 1943. Freehand tangential section below sorus, through patch of green tissues surrounding the sorus, from a leaf that had turned wholly yellow. After treatment for 4 days in the molybdenum reagent at 5° C. the nuclei (*n*) showed deep blue, as phospho-molybdenum blue had formed from the phosphorus compounds. The chloroplasts had retained their green color, showing that the chromo-proteins in these plastids were still intact, and did not release the compounded phosphorus. The large spherical coacervates (*c*) appeared deep brown red as the result of the oxidation of the phenolic compounds therein through the agency of the molybdenum reagent. Haustoria in cells (*ha*) showed a faint blue color due to phospho-molybdenum blue; the intercellular hyphae (*h*) are almost void, showing only a faint bluish coloration of the thin cytoplasm; it, therefore, appears that whatever phosphorus compounds had been obtained from the host had been translocated to the few urediospores formed (not featured in the figure). This section shows the coacervates as entirely different and independent from either the haustoria or the nuclei.

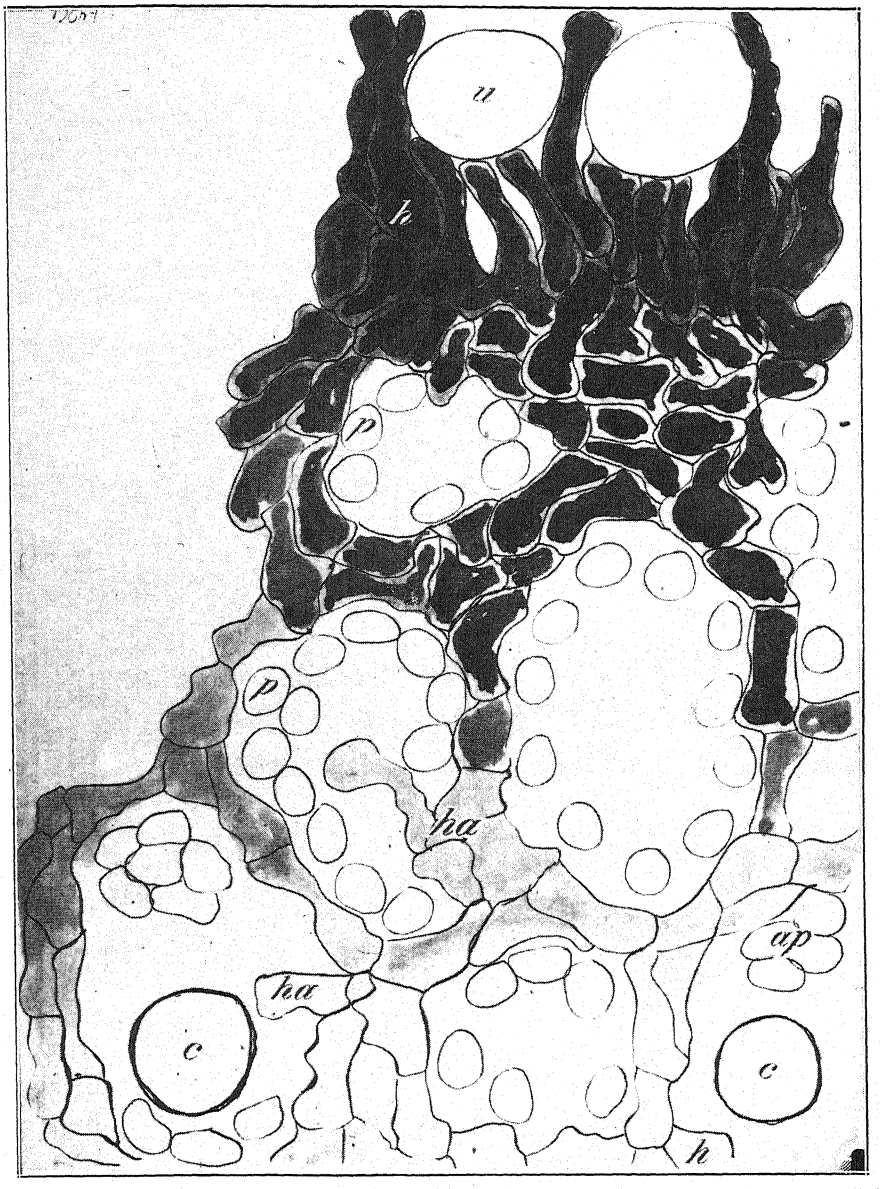


FIG. 6. Bond, inoculated January 14 with urediospores of race 1 of *Puccinia coronata* collected from Bond; examined February 5 in the molybdenum reagent. The hyphae (*h*) showed a gradient of intensity of development of the blue phospho-molybdenum reagent, as featured by the intensity of grey. The two urediospores (*u*) at the top showed their natural orange-red due to the carotenoids (the phosphorus in the urediospores is firmly bound with cell constituents and is unmasked only after several days in the reagent). Each of the two cells at base contains a large coacervate (*c*), sharply outlined by its phosphatid envelope and each containing the coacervated phenolic compounds, which the oxidizing effect of the molybdenum reagent has stained a deep red (*p*). Chloroplasts in outline. The chloroplasts are represented by their outline only; they were retaining their natural green color; the phosphorus is so firmly bound in the nucleo-protein constituent of the chromo-protein that it can be unmasked only after several days in the reagent. Some chloroplasts (*ap*) become agglutinated. The nucleus in each cell has not been featured; the nucleo-protein in the nuclei does not bind the phosphorus so firmly as one finds it in the plastids, and the nuclear phosphorus would give a blue color after 24 hours' treatment in the reagent. Note again that coacervates (*c*) are entirely independent from haustoria (*ha*).

Conversely, through the process of coacervation, these colloids may become apportioned to a special inclusion within the vacuolar solution, in the vacuole itself (Fig. 4). Then, if a freehand section of tissue in which colloids have become coacervated is immersed in neutral red or any other vital dye, the stain accumulates in the coacervates, which then become manifest as bright-colored spherical bodies, each surrounded by a well-defined membrane and suspended in the vacuolar solution, which no longer contains any stainable colloid, and, therefore, comes out of the bath unstained.

Coacervates, such as occur in crown-rust-infected leaves of the oat plant, belong to what Bungenberg de Jong and Kruyt (11) classified as "autocomplex coacervates," where the central core of coacervated phenolic compounds is permanently separated from the vacuolar solution by a membrane of phosphatids. Such coacervates are stable; they can best be demonstrated by immersing freehand sections in the molybdenum reagent, where the coacervated phenolic compounds will be oxidized to red quinoids, while the phosphorus from the phosphatid membrane will yield phospho-molybdenum blue (13), sharply outlining the contour of the coacervate itself (Figs. 5 and 6). This blue can be deepened to black by subsequent treatment in 1 per cent aqueous haematoxylin. The section can then be dehydrated rapidly with absolute alcohol and mounted in euperal.

CONCLUSIONS

Establishment of the parasitic relationship between the leaf cells of the oat plant, on the one hand, and the invading hyphae of the rust fungus (*Puccinia coronata*), on the other, depends on the release of phosphorus compounds by the host cells, which thus make available to the parasite their phosphorus. This release may be—very probably is—correlated to increased permeability, but also depends on a marked degradation of the phosphorylated compounds, a process referred to by Haag (16) as "phospholyse." This attempted physical interpretation of a cytological phenomenon does, however, leave us still lacking necessary enlightenment as to the facts basic to our understanding of the host-parasite relationship.

Cytochemical examination of the affected tissues reveals changes that can best be described as secretion phenomena. These appear to be homologous to the phenomena in either plant or animal cells following any stimulus, physical, chemical, or pathological, such as might be induced by a parasite or other pathogenic agent.

In all cases the affected cell gives up to the surrounding medium some phosphorus compounds, which it would normally retain intact. In the cells of an oat plant affected by rust there occurs, concomitantly with the excretion of materials into the intercellular spaces, an internal secretion within the vacuolar solution itself—a secretion resulting in the coacervation of phenolic compounds, mostly pyridoxin.

Indophenol-blue-forming phenolic compounds, such as pyridoxin, are widespread in the vacuolar solution of cells of the oat plant, and most

abundant in the vacuoles of the guard cells and of the long epidermal cells in line with the stomata.

Coacervation of those phenolic compounds seems correlated with the dispersion of the nucleotides or phosphoproteids in the cell and to the resulting decompensation of respiration. The establishment of the host-parasite relationship entails a decompensation of respiration that may still permit the host cell to survive. If, on the other hand, the decompensation be so severe as to prove rapidly lethal, the rust fungus no longer behaves as a parasite but as a pathogen, inducing necrotic spots characteristic of hypersusceptibility.

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THE MECHANISM OF WILTING CAUSED BY FUSARIUM BULBIGENUM VAR. LYCOPERSICI[1]

DAVID GOTTLIEB

(Accepted for publication July 3, 1943)

The first important hypothesis on the nature of vascular wilts was formulated by Atkinson (3) in 1892. His investigations on cotton wilt demonstrated that the pathogen is a vascular parasite; and Atkinson postulated that the plants wilted because the fungus utilized the nutrients present in the tracheal fluid and thereby starved the host. This hypothesis was soon superseded by the plugging theory, which assumed that the wefts of mycelium observed in the vessels of the host caused wilting by hindering the movement of water to the upper parts of the plant (12, 34, 35, 48, 49, 54). Most of the recent investigators, however, report the very rare occurrence of mycelial wefts, and that the hyphae usually grow sparsely along the inner walls of the tracheae (1, 2, 6, 10, 19, 20). Even if occlusion did occur in some of the vessels there seemed to be sufficient lateral diffusion to keep the plant in a turgid condition (8, 11). Other types of occlusion, such as the formation of tyloses or gums in the xylem (4, 22, 23, 24, 37, 47), also have been suggested, but these may be secondary factors and not the primary cause of wilting. Similar materials also have been observed in bacterial wilts (27, 53). Another explanation for wilting in flax has been advanced by Tochinai (52), who suggested that *Fusarium lini* might produce enough carbon dioxide to form a gas pocket in the tracheae and thereby break the transpiration stream. Link (32) and Mann (33) ascribed the wilting of potatoes to an infection and necrosis of the root tissues, which limited the water-absorbing powers of the plant; but Tisdale (51) and Grossman (19) pointed out that, in flax, such necrosis takes place only after the plant is already dead. Vascular fungi may even cause wilting by destroying the mesophyll tissues of the leaves. According to van der Meer (53), van der Lek observed that *Fusarium tracheiphila* grew out of the vessels and attacked the mesophyll of the leaves of cucumber. Only after these tissues were attacked did the plant wilt.

The inadequacy of the foregoing explanations of wilting led to the development of the toxin[2] theory, now most generally accepted. It is based, for the most part, on the fact that toxins are produced by the different vascular fungi, when they are grown on synthetic nutrient media, and on the ability of these toxins to wilt excised shoots (2, 6, 9, 15, 19, 21, 30, 56). Vascular discoloration, which is a diagnostic character for these diseases, also has been

[1] Condensed from a thesis submitted to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Degree granted December 1942. Paper No. 2089, Scientific Journal Series, Minnesota Experiment Station. Assistance in the preparation of figure 2 was furnished by the personnel of the Works Project Administration, Official Project No. 165–71–1–124, Sponsored by the University of Minnesota (1942).

[2] In this paper the term toxin is used to indicate a substance produced by a living organism that is deleterious to a plant.

attributed to these fungi. Analysis of the toxic filtrates from cultures of vascular parasites has yielded conflicting results, and the toxic activity has been variously attributed to different components such as nitrates, enzymes, acids, and alcohols (4, 14, 15, 20, 21, 42, 43, 44, 57). The more recent studies of Schaffnit and Lüdtke (46), Ahmet (1), Grossman (19), and Elpidina (13) indicate that the toxic fractions of the fungal filtrates may be amines. The toxin theory has been strengthened by the recent studies of Gottlieb (18) on fusarial tomato wilt. He reported the presence of toxins in the tracheal systems of wilted plants and their absence in the fluids obtained from noninoculated turgid plants.

There have been many theories to explain the action of the toxins on the host plant. Van der Lek, according to Rudolph (44), attributes the discoloration of the vessels to the corrosive action of a toxin, while Hursh (25) points out a correlation between the browning and an inactivation of the toxin. On the other hand, Picado (41), who also believed that the discoloration is due to metabolic products of the fungus, distinguished between discoloring action and the wilting effect. Pethybridge (40) believed this discoloration due to two components: the innate color of the fungal hyphae and another factor that is independent of the parasite.

A more important problem is the role of the toxin in disturbing the water relations of the plant. Bewley (4) and Higgins (22) postulate the secretion of substances that act upon the living cells, these cells, in turn, producing the gums that are the direct cause of wilting. Zentmeyer (60), in contrast, considered the tyloses and gums of secondary importance to the direct action of the toxins in causing wilting. Another vital question still unsatisfactorily answered is the kind of tissue the toxin affects. Hursh (26) thought the toxins acted on the vascular tissue, where they were deposited or inactivated. When stems were placed in fungus-culture filtrates, some of the cells at the exposed surface collapsed, thereby interfering with normal transpiration. If the ends of these stems were then cut off and the seedlings placed in water, the plants recovered. According to Hursh, permanent wilting sets in only when considerable functional tissue has been destroyed. Supporting this interpretation are the observations recently made by Maclean and Walker (34). In wilts of potatoes caused by *Fusarium avenaceum, Fusarium solani,* and *Fusarium oxysporum,* they observed that some of the small xylem vessels had collapsed and interpreted the collapse as indicating a dissolving action by the fungus.

Various investigators have suggested that the toxin might act on the leaf. As early as 1921, Young and Bennet (58, 59) believed that the toxin acted on the cell membranes, rendering them less permeable. Thus, under conditions of high transpiration, not enough water could reach the tissues to enable them to retain their turgidity. A similar hypothesis was presented by Clayton (8), who stated that either an increase or decrease in permeability could account for the wilting. Pertinent data on this subject have been presented by Linford (31), in transpiration studies on pea wilt,

from which he postulated increased permeability in diseased tissues. Fisher (16) also suggested that wilting of tomatoes was due to permeability changes in the cell membranes. Thatcher (50) measured the permeability of plants that wilted in fungal filtrates, and found that an increase in permeability was correlated with the wilted condition. More direct evidence that the toxin acts on the leaf tissues in some plants has been presented by Dawson (11) in his work on the *Cephalosporium* wilt of daisies. The most prominent symptom of this disease prior to actual wilting is an intense yellowing of the leaves. Dawson was able to reproduce this condition in the cells of the leaf by detaching them from the plant and placing them in a filtrate from the culture of the parasite. The mesophyll and palisade cells became an intense yellow and the chlorophyll was disorganized. Ahmet (1) believed that the plant could adjust itself to the new water relations and remain turgid if the pathogen was not too virulent, or other conditions did not favor an acute wilt. An adjustment of this type, he postulated, would account for the ability of some infected plants to continue functioning for some time, as occurs in the chronic wilts.

The object of the present investigation was to ascertain the immediate cause and mechanism of wilting of tomatoes due to *Fusarium bulbigenum* var. *lycopersici* (Bruschi) Wr. and R. Four principal phases of the problem were studied: the extent and effect of plugging; the production of toxins by vascular pathogens and other fungi; the presence of toxins in the tracheae of infected plants; and the effect of such toxins on the physiology of the host.

EXPERIMENTAL STUDIES

Effect and Extent of Plugging

Although the plugging theory of wilting has been discarded by most of the current pathologists, there is little direct evidence that it plays no part. More conclusive evidence on this subject was sought by a histological study of the growth and development of the pathogen in the different plant parts and by a study of the movement of water through infected and noninfected stems.

Bonny Best tomato plants were inoculated with *Fusarium bulbigenum* var. *lycopersici* by the Wellman procedure (55), and wilting occurred about 2 weeks later. Noninoculated plants were used as checks. Sections of tissue from the roots, 1 inch below the ground line, and from the stem at 1 inch and 9 inches above the ground and 1 inch below the uppermost petiole, were plated out on potato-dextrose agar. Fusarium cultures were isolated from the roots of 61 of the 63 inoculated plants, from 63 of the stem sections that were cut 1 inch above the ground line, and from 39 of the stem sections cut 9 inches above the ground line. No Fusarium species were ever isolated from the region of the uppermost petiole, indicating that any action of the fungus to cause wilting must begin at a distance from the leaf, the site of actual wilting. Twenty-one of the isolates from the roots and stems were

tested for pathogenicity, and all of them caused wilting in the inoculated tomato plants. From similar tissue sections of the check plants only 2 colonies of *Alternaria* were obtained that were considered as contaminants and unrelated to the disease.

At the time of wilting, half-inch sections of each of the above mentioned tissues were washed and fixed in formic acid, acetic acid, and alcohol solu-

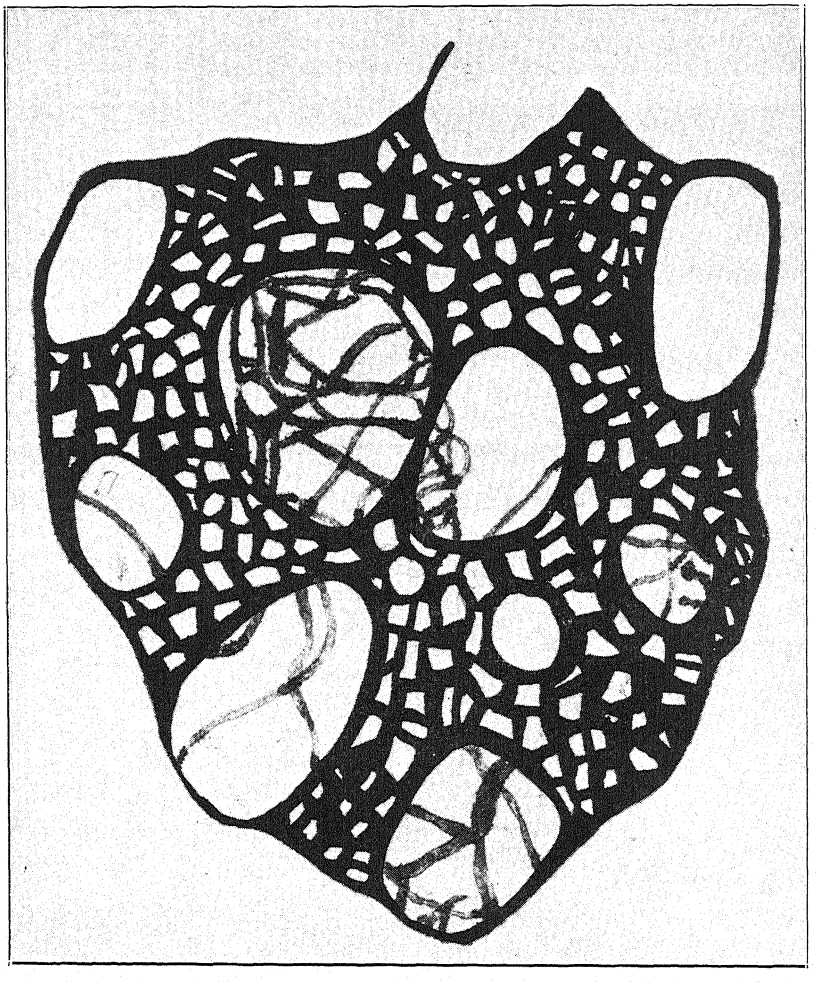


FIG. 1. Camera-lucida drawing of cross section of vascular bundle in lower portion of tomato stem infected with *Fusarium bulbigenum* var. *lycopersici*.

tion. Hand sections were made and the vascular tissues were studied microscopically. These studies confirmed the results of the culture studies, for all of the inoculated plants had mycelium in the lumen of the larger vessels of the roots and in the two lower portions of the stem, but none near the top of the shoot. No mycelium was present in any portion of the check plants. Approximately 45 per cent of the large vessels in any stem section contained

mycelium, although in some sections this ranged as high as 61 per cent. No plugging was observed in any of the sections made 9 inches above the soil line. But in the lower stem and in the roots, some of the vessels were well plugged with a mass of anastomosing and criss-crossing hyphae, occupying almost the entire cross-sectional area of the vessel (Fig. 1). Many of the

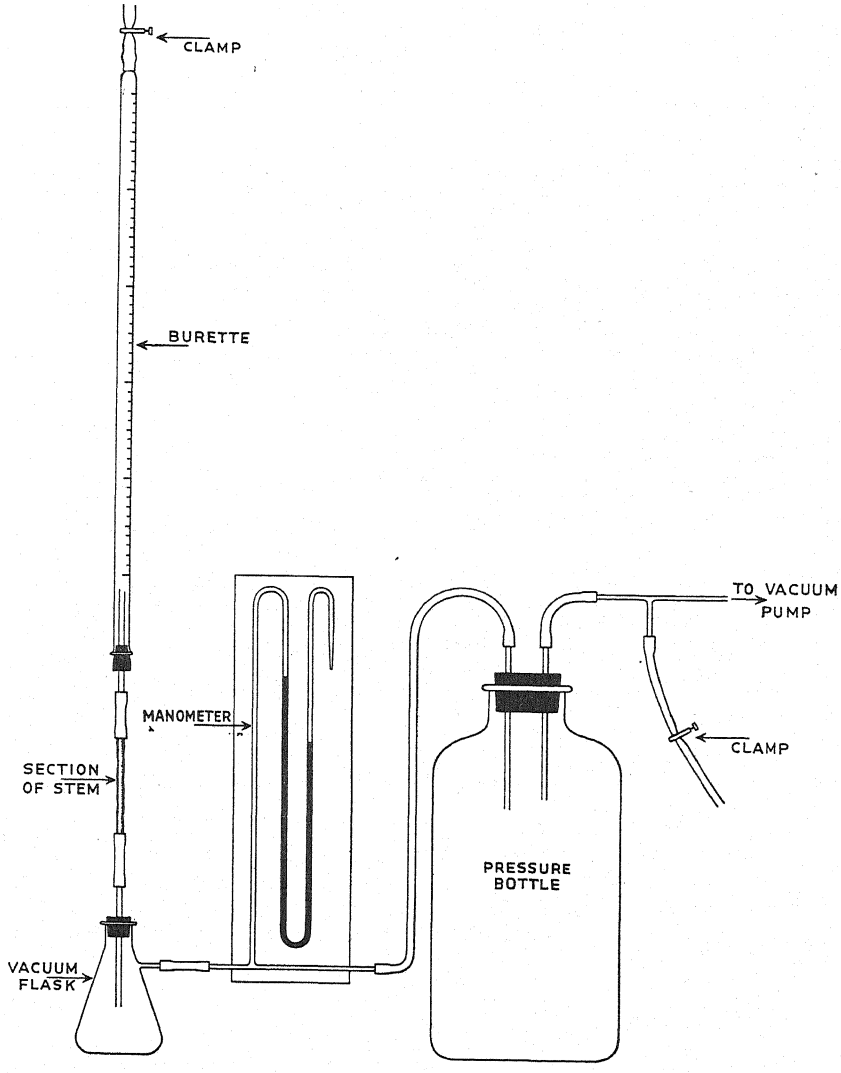


FIG. 2. Flowmeter for measuring the movement of fluids through stems.

vessels, however, contained only a few hyphae that formed a loose weft in the lumen. When one considers the length of tracheae that might thus be filled, it is apparent that the xylem could become a very inefficient transport organ. Nevertheless, even if the occluded vessels become nonfunctional, the remaining ones might still transport sufficient water to maintain turgidity of the plant.

Because many of the vessels were plugged, attempts were made to measure the movement of liquids through infected and noninfected stems. The movement of water through Fusarium-infected stems should be hindered if the obstruction by mycelium were responsible for wilting.

A flowmeter, therefore, was designed to measure the rate of flow of water through a section of stem (Fig. 2). An inverted burette containing water was connected to a section of the stem, which had been cut under water, by a short length of glass tube; and the lower end of the glass tube was then connected to another glass tube leading into the vacuum flask. The flask in turn was connected by suitable tubing to a mercury manometer and vacuum pump. A large bottle between the pump and the flask acted as a reservoir to cushion any changes in pressure, and an extra outlet from the T tube was used to regulate the pressure. Any length of stem could be accommodated by raising or lowering the clamp that held the burette. The stems were covered with petrolatum and a pressure of 13 cm. less than atmospheric was applied to the stem. This gave a flow of about 0.5 ml. of water in 30 min. through a 15-cm. section. Repeated trials demonstrated the precision of the apparatus. For example, 5 trials on a stem section of a noninoculated plant gave the following results: 1st run, 0.48 ml.; 2nd run, 0.46 ml.; 3rd run, 0.49 ml.; 4th run, 0.47 ml.; 5th run, 0.47 ml.

Upon further investigation it was discovered that the procedure could not be used for diseased tomatoes. The lower portions of the stems usually were hollow because of the disintegration of the pith, a condition that seemed more severe in infected than in normal plants. Thus, results would not be comparable even when stems of the same size were used. Although the upper portion of the stem was still solid, it could not be used because it contained very little mycelium. Therefore, no conclusions could be drawn as to the effect of plugging on the movement of water through diseased stems.

TOXINS IN CULTURE

The production of toxins in culture by vascular parasites has been the principal evidence supporting the toxin theory of wilting. But such evidence has not been considered entirely conclusive, since the metabolism of microorganisms may vary with the culture medium (5). Rosen (43), for example, showed that the ability of toxins from *Fusarium vasinfectum* to cause wilting in cotton plants depends on the culture medium on which the fungus has been grown. Lathrope (28) thought that the aldehydes in the filtrates of cultures of *Fusarium cubense* were responsible for wilting in the host, but he was unable to find such compounds in the diseased plants. Various fungi other than vascular parasites also produce toxins. It was, therefore, interesting to test a large variety of fungi and determine their ability to produce wilt toxins.

Twenty-six fungi including vascular parasites, root-rotting organisms, leaf-spotting fungi, and saprophytes (Table 1) were studied. All the fungi were grown in Richards' solution, except *Ustilago zeae,* which was grown in

potato-dextrose broth. Fifty milliliter aliquots of the nutrient solutions were placed in Erlenmeyer flasks and inoculated with approximately equal amounts of the organisms. Two series were run, a short series of 17 fungi, which had been grown at room temperature for 2 weeks, and a long series of 26 fungi, which had grown for 4 weeks. At the end of that time each culture was filtered and the filtrates were poured into 10-ml. vials into which 21-day-old tomato seedlings were placed. Six seedlings were used for each filtrate and all were illuminated by a 100-watt lamp 18 inches above the table. Four of the 5 vascular parasites in the "2-week cultures" produced

TABLE 1.—*Wilt reactions of tomato seedlings in filtrates of various fungi*

Organism	Reaction to filtrate from	
	2-week culture	4-week culture
Fusarium lini	Wilted	Wilted
Fusarium eumartii	Turgid	Turgid
Fusarium conglutinans	Wilted	Wilted
Fusarium elegans	.....	Wilted
Fusarium niveum	Wilted	Turgid
Fusarium lycopersici	.....	Wilted
Fusarium pisi	.....	Turgid
Verticillium albo-atrum	Wilted	Wilted
Fusarium gramineum	.....	Turgid
Papulospora sp.	Wilted	Wilted
Helminthosporium avenae	Wilted	Wilted
Helminthosporium sativum	Turgid	Turgid
Aspergillus clavatus	Turgid	Turgid
Aspergillus ochraceus	Wilted	Turgid
Aspergillus niger	Wilted	Turgid
Perconia byssoides	Wilted	Wilted
Styansus stemonitis	Wilted	Wilted
Penicillium expansum	Wilted	Wilted
Cladosporium herbarum	Turgid	Turgid
Colletotrichum phomoides	Turgid	Wilted
Cephalosporium roseum	Wilted	Wilted
Arachniotus trisporus	.....	Wilted
Arachniotus sp.	.....	Turgid
Rhizoctonia solani (isolate 1)	.....	Wilted
Rhizoctonia solani (isolate 2)	.....	Turgid
Ustilago zeae	.....	Wilted

toxins, while 5 of the 8 in the "4-week cultures" produced them. Eight of the 12 remaining fungi in the "2-week cultures" produced toxins, while 10 of 18 fungi in the "4-week cultures" produced them (Table 1). Thus, it is evident that many fungi can produce toxins that cause wilting in tomato seedlings, when such fungi are grown on artificial media. An examination of table 1 shows that even different isolates of the same species may vary in toxin production; one isolate of *Rhizoctonia solani* produced toxins and the other did not. The toxicity of the filtrate also may vary with the age of the culture, as in the case of *Colletotrichum phomoides*. The 2-week-old culture, for example, was unable to cause wilting, whereas the "4-week cul-

tures'' caused wilt of all 6 seedlings. The results obtained with *Aspergillus* sp., however, were difficult to interpret because 2 of the fungi produced toxins in the ''2-week cultures,'' but did not produce them in the ''4-week cultures.'' This discrepancy might be explained by differences in the test seedlings. Hursh (26) found that the wilting ability of toxins will vary with the age of the plant they act upon, hardiness of the stem, and other factors. It is possible that the seedlings used in filtrates from the ''4-week cultures'' were hardier than those used for the ''2-week cultures,'' although an attempt was made to employ a uniform group of seedlings in all tests. Despite these discrepancies, the experiment indicates that the production of toxins in culture is in itself not sufficient evidence that toxins are produced in plants infected with vascular parasites. The evidence that such deleterious substances cause wilting would be greatly strengthened if their presence could be demonstrated in the wilted plants.

TOXINS IN TRACHEAL FLUIDS

In view of the fact that the results reported in a previous paper (18) had indicated the presence of deleterious substances in the tracheal fluids of wilted plants, it was thought important to repeat these experiments and make further studies on tracheal fluids. Two hundred Bonny Best tomato plants were inoculated by the Wellman technique (55) when about 18 inches high. An equal number of plants were grown as checks under the same conditions, except that they were transplanted without inoculation. The inoculated plants wilted in about 3 weeks, while the noninoculated checks remained turgid. All the plants were then extracted by the technique and apparatus described previously (18). Thirty-five seedlings were used in the assay, 15 for the fluids from wilted plants, 15 for the fluids from the turgid plants, and 5 as absolute checks in distilled water. All the seedlings in the ''wilt fluids'' lost turgidity within 2 hours, whereas those in the ''normal fluids'' and in distilled water remained turgid, even after 12 hours (Table 2). Further studies also were made on plants that had wilted because of insufficient soil moisture, but their extracts exerted no deleterious effects on the test seedlings.

Similar studies also were made on plants that had chronic wilt, in which there is not a sudden loss of turgidity. Instead, the leaves gradually yellow in basipetalous successions and eventually become brittle. Sometimes the plant does not die but remains dwarfed and unthrifty. This condition usually occurs when the environment is unfavorable for the disease, when the host is somewhat resistant, or when the pathogen is not virulent. An assay for deleterious substances was made on the vascular fluids of such plants. The plants and the pathogen were handled in the usual manner, except that the temperatures were somewhat lower, about 65° C. rather than 80° C., and the soil was kept saturated. The checks were grown under similar conditions. The typical chronic wilt appeared in the inoculated plants,

which were then used for the extraction. Sixteen seedlings were used in the assay, 6 for the wilt fluid, 6 for the normal fluid, and 4 for distilled water. Five of the 6 seedlings in the wilt fluid became flaccid after 2.5 hours; the remaining one was only slightly wilted, even after 12 hours. All the plants in the normal fluids and in water remained turgid for at least 12

TABLE 2.—*Time required for the wilting of seedlings in tracheal fluids from tomatoes inoculated with Fusarium bulbigenum var. lycopersici and from noninoculated plants*

Fluid[a]	Condition of seedlings[b] from 1 to 12 hours after placement in fluid											
	1	2	3	4	5	6	7	8	9	10	11	12
WF_1	SW	W	W	W	W	W	W	W	W	W	W	W
WF_2	SW	W	W	W	W	W	W	W	W	W	W	W
WF_3	SW	W	W	W	W	W	W	W	W	W	W	W
WF_4	SW	W	W	W	W	W	W	W	W	W	W	W
WF_5	SW	W	W	W	W	W	W	W	W	W	W	W
WF_6	T	SW	T	W	W	W	W	W	W	W	W	W
WF_7	T	SW	T	W	W	W	W	W	W	W	W	W
WF_8	T	SW	T	W	W	W	W	W	W	W	W	W
WF_9	T	SW	T	W	W	W	W	W	W	W	W	W
WF_{10}	T	SW	T	W	W	W	W	W	W	W	W	W
WF_{11}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{12}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{13}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{14}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{15}	T	T	SW	W	W	W	W	W	W	W	W	W
NF_1	SW	T	T	T	T	T	T	T	T	T	T	T
NF_2	SW	T	T	T	T	T	T	T	T	T	T	T
NF_3	SW	T	T	T	T	T	T	T	T	T	T	T
NF_4	SW	T	T	T	T	T	T	T	T	T	T	T
NF_5	SW	T	T	T	T	T	T	T	T	T	T	T
NF_6	T	T	SW	T	T	T	T	T	T	T	T	T
NF_7	T	T	T	T	T	T	T	T	T	T	T	T
NF_8	T	T	SW	T	T	T	T	T	T	T	T	T
NF_9	T	T	T	T	T	T	T	T	T	T	T	T
NF_{10}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{11}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{12}	T	T	T	SW	T	T	T	T	T	T	T	T
NF_{13}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{14}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{15}	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_1	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_2	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_3	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_4	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_5	T	T	T	T	T	T	T	T	T	T	T	T

[a] WF indicates fluids from wilted plants; NF, fluids from normal noninoculated plants; H_2O, water checks.
[b] T, turgid; SW, slightly wilted; W, wilted.

hours (Table 3). From these results we can conclude that a toxin is present in the tracheal fluids of tomatoes infected with *Fusarium bulbigenum* var. *lycopersici* and that it is definitely associated with the presence of the pathogen. Since the toxins are also present in chronically wilted plants, Ahmet's (1) hypothesis that the host may adjust itself somewhat to the presence of the pathogen seems tenable.

Properties of the Toxins

Three properties of the toxins were investigated; the stability in storage, the ease of oxidation, and the thermolability. Vascular fluids that had been stored under nitrogen at 18° C. did not lose their toxicity for tomato seedlings, even after 120 days. The oxydizability of the toxin was determined by bubbling a stream of oxygen through 3 1-ml. samples of "wilt fluids" for 2 hours. "Normal fluids" also were saturated with a stream of oxygen to ascertain whether any toxic compounds were formed during this process. Untreated "wilt fluids" were used as checks and no significant changes

TABLE 3.—*Time required for wilting of seedlings in tracheal fluids of chronically wilted plants*

Fluid[a]	Condition of seedlings[b] from 1 to 12 hours after placement in fluid											
	1	2	3	4	5	6	7	8	9	10	11	12
WF_1	T	W	W	W	W	W	W	W	W	W	W	W
WF_2	T	T	W	W	W	W	W	W	W	W	W	W
WF_3	T	W	W	W	W	W	W	W	W	W	W	W
WF_4	T	SW	SW	SW	SW	SW	SW	SW	SW	SW	SW	SW
WF_5	T	W	W	W	W	W	W	W	W	W	W	W
WF_6	T	T	T	W	W	W	W	W	W	W	W	W
NF_1	T	T	T	T	T	T	T	T	T	T	T	T
NF_2	T	T	T	T	T	T	T	T	T	T	T	T
NF_3	T	T	T	T	T	T	T	T	T	T	T	T
NF_4	T	T	T	T	T	T	T	T	T	T	T	T
NF_5	T	T	T	T	T	T	T	T	T	T	T	T
NF_6	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_1	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_2	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_3	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_4	T	T	T	T	T	T	T	T	T	T	T	T

[a] WF indicates fluids from wilted plants; NF, fluids from noninoculated plants; H_2O, water checks.
[b] T, turgid; SW, slightly wilted; W, wilted.

resulted from oxygenating the wilt fluids. All the seedlings in both the treated and the nontreated wilt fluids wilted within 1.5 hours, whereas those in the oxygenated normal fluids remained turgid even after 12 hours.

The results of the temperature studies were more difficult to interpret. "Wilt fluids" and "nontoxic fluids" were placed in test tubes that were then filled with nitrogen and sealed. Some of the tubes were heated to 100° C., others to 250° C. for 15 minutes; still others were held at room temperature to serve as checks. The tubes, heated to 100° C., contained a fine suspension that did not settle out on standing, while in the tracheal fluids, which had been heated to 250° C., there was much greater denaturation and a copious precipitate. Only the clear supernatant liquid of the latter was used in the assay. Both heated liquids had a sour odor, which was very strong in the tubes that had been heated to 250° C. There were no consistent differences in the time for wilting of seedlings in the fluids that had been heated to 100° C. and the checks; wilting occurred within 1

hour. But one could not make certain that the original toxin was stable and had caused the wilting because the colloidal suspension present might have precipitated in the vessels of the seedlings and plugged them. The supernatant liquid from tracheal fluids that had been heated to 250° C. did not bring about wilting of the test seedlings, whereas the non-heated fluids wilted tomato seedlings within 2 hours.

Because various investigators had indicated that inorganic chemicals may be the toxic components of the tracheal fluids of diseased plants, the tracheal fluids were analyzed spectrographically and the emission element content compared to those of normal plants. Four replicates of each fluid were analyzed but no perceptible differences for any of the emission elements were obtained.

THE PHYSIOLOGY OF WILTING

Many field observations indicate a correlation between the prevalence of wilt and hot dry weather, which tends to increase transpiration. But there are very few reports available in which the transpiration of the diseased plants has been controlled for experimental study. In the present investigations transpiration was controlled in two ways: first, by mechanically preventing evaporation; and second, by the use of a saturated atmosphere. The mechanical prevention of transpiration was accomplished by coating all the exposed surfaces of the test seedlings with vaseline before placing them in the vials containing the wilt fluids. Twenty seedlings were coated in this manner and 20 noncoated ones were used as checks. All the seedlings were placed under electric lamps, at a temperature of 32° C. and a relative humidity of 52 per cent. None of the treated seedlings wilted within 18 hours, whereas the nontreated seedlings lost turgidity within 2 hours. In other experiments seedlings were placed in a saturated atmosphere over a layer of water in a desiccator. Fifteen of the seedlings in the toxic fluids were placed in the moist chamber and 15 were left exposed on the laboratory table; both sets were illuminated by two 100-watt bulbs, which gave an illumination of 405 foot candles. The temperature varied between 26° and 28° C. None of the seedlings in the desiccator wilted, while all those that were exposed in the rather hot, dry air became limp (Table 4). To further test the efficacy of a saturated atmosphere in preventing wilt, 10 seedlings that had wilted under conditions of low humidity were placed in a moist chamber while still in the toxic tracheal fluids and 10 were left in the same environment that had brought on the wilting. All seedlings in the moist chamber recovered within 12 hours, while the others dried without ever regaining turgidity. When the recovered seedlings were removed from the moist chamber to the table, wilting recurred. If again placed in the humid environment, recovery followed; and a subsequent removal again resulted in a loss of turgidity.

Similar experiments were made in the greenhouse with plants that had been inoculated with *Fusarium bulbigenum* var. *lycopersici*. The seedlings

were grown in flats and inoculated when they were about 5 weeks old, after which they were immediately transplanted to 4-inch pots. They were kept in the shade for a week to allow for recovery from the injury of transplanting, then placed on the greenhouse bench. Twenty-four of the pots were placed under bell jars and 25 were left on the bench uncovered. In addition 10 noninoculated seedlings were placed under bell jars as checks. All plants were watered daily, and those under bell jars had large guttation drops hanging from the leaves because of the humid conditions. Of the

TABLE 4.—*Time required for wilting of seedlings in toxic tracheal fluids under different humidities*

Atmosphere	Fluid[a]	Condition of seedlings[b] from 1 to 10 hours after placement of fluids									
		1	2	3	4	5	6	7	8	9	10
Unsaturated atmosphere	WF_1	T	W	W	W	W	W	W	W	W	W
	WF_2	W	W	W	W	W	W	W	W	W	W
	WF_3	W	W	W	W	W	W	W	W	W	W
	WF_4	T	T	W	W	W	W	W	W	W	W
	WF_5	W	W	W	W	W	W	W	W	W	W
	WF_6	W	W	W	W	W	W	W	W	W	W
	WF_7	W	W	W	W	W	W	W	W	W	W
	WF_8	T	W	W	W	W	W	W	W	W	W
	WF_9	T	T	W	W	W	W	W	W	W	W
	WF_{10}	T	T	W	W	W	W	W	W	W	W
	WF_{11}	W	W	W	W	W	W	W	W	W	W
	WF_{12}	T	T	W	W	W	W	W	W	W	W
	WF_{13}	T	W	W	W	W	W	W	W	W	W
	WF_{14}	W	W	W	W	W	W	W	W	W	W
	WF_{15}	W	W	W	W	W	W	W	W	W	W
Saturated atmosphere	WF_{16}	T	T	T	T	T	T	T	T	T	T
	WF_{17}	T	T	T	T	T	T	T	T	T	T
	WF_{18}	T	T	T	T	T	T	T	T	T	T
	WF_{19}	T	T	T	T	T	T	T	T	T	T
	WF_{20}	T	T	T	T	T	T	T	T	T	T
	WF_{21}	T	T	T	T	T	T	T	T	T	T
	WF_{22}	T	T	T	T	T	T	T	T	T	T
	WF_{23}	T	T	T	T	T	T	T	T	T	T
	WF_{24}	T	T	T	T	T	T	T	T	T	T
	WF_{25}	T	T	T	T	T	T	T	T	T	T
	WF_{26}	T	T	T	T	T	T	T	T	T	T
	WF_{27}	T	T	T	T	T	T	T	T	T	T
	WF_{28}	T	T	T	T	T	T	T	T	T	T
	WF_{29}	T	T	T	T	T	T	T	T	T	T
	WF_{30}	T	T	T	T	T	T	T	T	T	T

[a] WF indicates tracheal fluids obtained from diesased wilted plants.
[b] T denotes turgid and W denotes wilted.

25 tomato plants exposed on the greenhouse bench, 7 wilted in ten days, 5 more in 12 days, and the remaining within 14 days. All the inoculated plants under the bell jar remained turgid for 28 days. Thereafter, they gradually wilted; and at the end of an 8-week period only 4 of the original 24 plants were still turgid. But, even the tomato plants that survived had an unthrifty appearance, and when removed from the bell jar to the greenhouse bench they too died. Although none of the noninoculated plants under the bell jars wilted, they also appeared unthrifty and the waterlogged soil and saturated atmosphere seemed detrimental to their vigor.

From these results it is apparent that the toxin disturbs in some way the relation between the water intake of the plants and their loss of water by transpiration. As long as transpiration is reduced the cells do not lose their turgor. The fact that even those inoculated plants that had been kept under bell jars wilted eventually might be explained by the general detrimental effect of the water-logged soil and saturated atmosphere on the vigor of the plants. Noninoculated plants also did not thrive under similar conditions. Thus, the combined effect of the presence of toxin and adverse environment may have brought about the death of inoculated plants.

Toxins and Permeability

As suggested by some earlier investigators, toxins resulting from an infection by *Fusarium bulbigenum* var. *lycopersici* might alter the permeability of the host cells, thereby upsetting the water balance of the host. Experiments were, therefore, set up to measure such changes in cell permeability. Tracheal fluids were obtained from inoculated and noninoculated

TABLE 5.—*Time of deplasmolysis for cells in tissue sections placed in tracheal fluids from infected and noninfected plants*

Fluid[a]	Minutes	Fluid[a]	Minutes
IWF_1	3	NWF_1	7
IWF_2	4	NWF_2	5
IWF_3	4	NWF_3	4
IWF_4	4	NWF_4	5
IWF_5	4	NWF_5	17
IWF_6	5	NWF_6	17
IWF_7	4	NWF_7	14
IWF_8	4	NWF_8	17

[a] IWF denotes tracheal fluids of infected plants. NWF denotes fluids of plants wilted because of insufficient soil moisture.

plants in the usual manner, and fluids of normal noninoculated plants that had wilted because of lack of soil moisture were used as absolute checks. The latter control was important; otherwise, any changes in the permeability of the infected, wilted plants, when compared to the permeability of normal turgid plants might be due to the process of wilting itself and have no relation to the presence of the parasite in the vascular tissue.

All measurements of changes in permeability were made on the pith cells from petioles of actively growing young plants. Using calcium chloride solutions of various concentrations, the osmotic pressure of normal noninoculated plants was determined as 10.6 atmospheres, which was equivalent to a 0.403 molar solution isotonic with the pith cells. A hypertonic 0.806 was used for plasmolysis and a hypotonic 0.303 molar solution for deplasmolysis, and the time necessary for deplasmolysis was used as a criterion of cell permeability. Since absolute permeabilities would give no information pertinent to the problem, these values were not calculated. Tissue sections were placed in the tracheal fluids from both infected and noninfected plants for 2 hours and then removed to the hypertonic solution

for 20 minutes. After removing the excess of reagent, the sections were placed in the isotonic solution and observed for time of deplasmolysis. At least 100 cells were observed in each section. All but one of the sections, IWF_6, that had been placed in the toxic fluids, required less time for deplasmolysis than those in the normal fluids (Table 5). The results of the foregoing experiments indicate that the toxin produced in infected tomato plants increases the permeabilities of the cells. Such an increase in permeability could readily play an important role in causing tomato plants infected with the *Fusarium* to wilt.

PERMANENCE OF THE TOXIC ACTION

Although wilting was reversible when conditions that hindered transpiration prevailed, these results do not indicate whether or not a permanent change in permeability had taken place. For this reason a series of experiments was made in which plants were removed from the toxic fluids at various periods after wilting had begun, and placed in distilled water. The periods of time after wilting set in varied from 15 min. to 5 hr. All the plants recovered except those that had already started to dry out, which indicated a permanent disorganization of the tissues. Under the conditions of the experiment the maximum time that seedlings could remain in the wilted state and still recover turgidity was 4.25 hours. Hursh (25) reported that after wilted cabbage seedlings had recovered in water they did not again wilt when returned to the toxic filtrates, and Chester (7) interpreted this phenomenon as an example of acquired resistance in plants. Similar experiments were performed with tomato seedlings, both in fungus-culture filtrates and in toxic tracheal fluids. Twenty seedlings that had wilted in the tracheal fluids and recovered in the water within 4 hours again lost their turgidity when returned to those toxic fluids. Thirty-nine seedlings responded in a corresponding manner to toxic filtrates from fungus cultures. In no case was there any evidence of acquired resistance in the tomato seedlings.

Attempts were made to discover whether plants infected by the pathogen will recover when the supply of toxin is interrupted. Plants that had been inoculated and grown in the greenhouse until wilting began were cut a few inches below the uppermost petiole and the tops placed in water. At this period the upper portion of the stem contained no mycelium, and thus the top could obtain no more toxin during the time it was in the water. All these plants recovered. But those plants whose stems were cut at the soil line and then placed in water never recovered. The fungus was well established in the lower portion of the stem and could therefore continue to produce more toxin, which resulted in the death of the plant. The recovery of the tomato plants when removed from the influence of the fungus or from the toxic filtrate indicates that the toxin itself probably does not permanently disorganize the cell. The plant dies only after the disorganization of the water relations has proceeded far enough to bring about other changes

in the physiology of the host. If the effect of the toxin is removed before that time, the tissues regain their normal function. An uninterrupted toxin supply seems necessary before permanent disorganization of the protoplast occurs.

DISCUSSION

Although toxins long have been suspected of playing an important role in vascular wilts, there has been no proof hitherto of their presence in the tissues of infected plants. The results of the present investigation and of a previous one by the writer, demonstrated such deleterious substances in the tracheal fluids of tomato plants wilted by *Fusarium bulbigenum* var. *lycopersici*. Moreover, the active constituent is definitely associated with the presence of the pathogen in the vascular tissues, for no toxins could be obtained in noninoculated plants that wilted because of lack of soil moisture. It was not possible, however, to determine whether the toxin is a metabolic product of the fungus or a reaction product of the host due to the presence of the fungus. In addition to causing wilting of inoculated plants the toxin apparently brings about a discoloration of the xylem similar to that observed by Rumbold (45) when deleterious substances were injected into trees. Such symptoms also were observed by Overton (39) and Haskell (20) when stems were injured by steam, in which case Overton postulated that the toxins were a product of the dead cells. In the present investigation the toxins seem to be carried from the infected portion of the xylem to the leaves by the transpiration stream, as has been demonstrated by Free (17) for toxic minerals from the soil, because of the evaporation of the transpired water from the leaves. Free believed that the concentration of the toxin is increased until a derangement of the cell takes place.

The toxins in tomato appear to disturb the water balance of the host plant. Normally, there is a critical balance between the intake and the loss of water by the plant. A similar ratio must also exist in the leaf tissues, so that wilting can occur either because of increased transpiration or decrease in absorption of water. The toxins produced in the plant would tend to reduce that ratio below the critical level. Apparent wilting may conceivably be due also to a loss of turgidity as a result of the excretion of water from living cells into the intercellular spaces. However, wilting never occurred in tomato plants when they were in the toxic fluids, provided transpiration was prevented; and plants that had previously wilted regained their turgidity under such conditions. These results help explain the prevalence of vascular wilts during hot, dry periods when the transpiration is so high and the absorption so low that the upset in water relations of the plant is easily carried beyond the point necessary for turgidity. They also may explain the diurnal wilting and recovery reported in diseased plants. These hosts might wilt in the daytime as a result of the accelerating effect of the toxin on the movement of water out of the leaves; they recover their turgidity in the night when the transpiration is decreased so that the absorption transpiration ratio is raised above the critical level. This peri-

odic wilting lasts only a few days because in infected plants the parasite constantly produces toxin, which eventually could reduce the ratio so much that even the nightly decrease in transpiration would not suffice to raise it above the critical level. Somewhat analogous conditions were obtained experimentally when wilted tomato seedlings were allowed to remain in toxic filtrates for a period of 5 hours; when transferred to the moist chamber the seedlings did not regain turgidity, whereas those plants that were transferred sooner recovered temporarily.

The results obtained by measuring cell permeabilities indicate that the toxins present in the tracheal fluids of diseased tomato plants increase the permeability of the cell to water. These results are in agreement with the observations of Thatcher (50) on tomato plants that had wilted in "fungal filtrates." He observed a small increase in permeability for cells that had wilted when compared to the cellular permeability of turgid plants. There is a possibility that the increased permeability he observed may have been the result of the process of wilting and not its cause, for similar processes, such as drought hardening, also increase cell permeability (29). In the current investigation, to overcome this difficulty, small sections of healthy tissue were immersed immediately in plant fluids where wilting was impossible; and comparisons of cell permeabilities were between those placed in tracheal fluids from diseased plants and those placed in fluids from plants wilted because of insufficient soil moisture. Because of the increased transpiration at the time of wilting of pea plants, infected with *Fusarium pisi,* Linford (31) also postulated an increased permeability in the fusarial wilt of peas. Yet, his data indicated that, prior to actual wilting, there is a reduction in transpiration. Such a reduction might not necessarily indicate that there is also an initial decrease in permeability, for it is known that incipient wilting is accompanied by a loss of turgor that would cause the guard cells to close (38) and thereby reduce transpiration. When true wilting occurs the guard cells open wide and transpiration sharply increases (36).

The recovery of wilted seedlings when placed in water can be accounted for by the assumption of an inactivation of the toxin by the tissues. If the activity of the toxin permanently changed the permeability of the cell membranes the transference of wilted plants to water should not result in their recovery. Since the plants do regain their turgidity, the effect of the toxins is not lasting; and one might assume that the toxins that caused the initial wilting may have been inactivated or combined in some manner in the host cells. A continuous supply of toxin is, therefore, necessary to keep the plant in a wilted condition. This condition results when seedlings are kept in the toxic tracheal fluids and when the pathogen in the infected plants continuously produces the toxins that are transported to the leaves.

Very little is known concerning the nature of the tracheal toxins other than their stability to oxygen and perhaps to high temperatures. Spectrographic analyses have shown no correlation between the emission element

content of the tracheal fluids and the toxicity of these fluids to tomato seedlings. Greater promise for determining the toxic fraction lies in the field of bio-organic chemistry. Special attention should be given to the nitrogen, and amine content of the toxic and nontoxic tracheal fluids to establish whether the toxic amine fraction found by Ahmet (1) and Grossman (19) in the "fungal filtrates" is also present in the tracheal fluids of diseased tomato plants.

SUMMARY

A dense mycelial growth of *Fusarium bulbigenum* var. *lycopersici* was found in approximately 50 per cent of the vessels in the root and lower stem of wilted tomato plants, but no isolations of the fungus could be made from the top of the stem.

Attempts to measure the effect of this occlusion on the movement of water through tomato stems were unsuccessful.

Toxins are produced in culture by a large variety of fungi, including vascular parasites, root-rotting and leaf-spotting organisms, as well as saprophytes.

The wilting of tomato plants infected with *Fusarium bulbigenum* var. *lycopersici* is correlated with the presence of a toxin in the tracheal fluid of the host. Infections that result in chronic wilt also bring about the production of toxins in the tracheal fluids.

The toxins obtained from diseased tomato plants are stable to oxidation, and spectrographic analysis revealed no significant differences between the emission-element content of fluids from the diseased and from healthy plants.

The toxin increases the permeability of cells of the host plant.

Plants that have been wilted by the toxic fluids will recover when placed in distilled water, while their subsequent removal to toxic fluids will again cause the loss of turgidity.

The toxin disturbs the normal water relations of the plant. When the transpiration of seedlings that had been placed in the toxic tracheal fluids is prevented, wilting does not occur. Even wilted seedlings eventually regain their turgidity under these conditions.

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ACUTE AND CHRONIC SYMPTOMS IN THE TOBACCO RING-SPOT DISEASE

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INTRODUCTION

Reports by Valleau (8), by McKinney (1) and by McKinney and Clayton (4) have emphasized that the tobacco ring-spot disease is characterized by two phases that may be regarded as being essentially acute and chronic, respectively. It has been reported also that the acute phase may be brief, followed by an extended chronic phase (1), or the acute phase may continue from infection to maturity of the plant (1); that these disease reactions are influenced by the age of the plant, the amount of inoculum introduced, and growth conditions (1, 4); that tobacco is a host of medium susceptibility (1); that Early White Spine cucumber is more susceptible than tobacco (4); that the chronic phase is attended by mosaic symptoms more regularly in Early White Spine cucumber than in tobacco (4); and that the causal agent may be regarded as a mosaic virus (4).

The fact that the chronic phase of the ring-spot disease in tobacco usually is not attended by striking visible symptoms has led to the view that the progress of the disease differs fundamentally from the progress of the mosaic diseases. However, this view is not supported by all of the facts. One of the writers, McKinney (1), has taken the view that natural resistance in the commercial tobaccos is the chief basis for the usual lack of striking symptoms in the leaves that appear during the chronic phase, and for the delay in the onset of the chronic phase when the older plants are inoculated.

These studies were carried out to provide information on several factors influencing disease expression, *i.e.*, age of plants, culture conditions, methods of inoculation, host genotype and host species. Limited tests were carried out to determine the effects of the virus on the weight of leaves or leaves and stems.

MATERIALS AND METHODS

The studies reported here were conducted chiefly with the following varieties and collections of tobacco; Wisconsin-Havana Seed, Samsun (Turkish), T.I. 448A from Colombia and T.I. 706 from Honduras. Most of the work with ring spot was done with T.I. 448A and other selections from this number for the reason that they are susceptible to it and are resistant to tobacco mosaic. This made it possible to avoid the troublesome confusion with the latter disease in the field.

Tobacco ring-spot virus supplied by W. C. Price was used throughout the studies. Inoculations were made by various methods indicated throughout the text. The most active virus was obtained from the leaves of infected cucumber, *Cucumis sativus* L. var. Early White Spine.

[1] Acknowledgment is due Matthew Koerner for assistance in conducting the tests.

Plants were cultured in the greenhouse, in pots, large pails, and large wooden boxes. In the field, the tests were conducted in the usual manner current in experimental work with tobacco. The soil was well suited to tobacco culture and a balanced fertilizer was applied at the beginning of the season.

Virus assays were conducted on plants of bean, *Phaseolus vulgaris* L., var. Scotia and cucumber, var. Early White Spine. Extracts were applied to primary leaves or on cotyledons by means of the carborundum-wipe method. Temperatures were maintained near 33° C. for 24 to 48 hours after inoculation. After this period the ordinary greenhouse temperatures were maintained.

Primary lesions tend to be irregular on both test plants and, therefore, most comparisons were based on the number of plants developing secondary, acute symptoms. Cucumber was favored as the test plant as it provided large quantities of active virus from the chronic mosaic leaves, whereas in the bean the systemic infection induces necrosis and death. These tissues and the primary lesion tissue yield less virus than the leaves from cucumber plants.

RESULTS ON TOBACCO

Influence of Age of Plants and Culture Methods on Symptom Expression

Early in the studies on the ring-spot disease, it was observed that irregularities occurred in symptom expressions from test to test. Many of these observations suggested that the age of the plants and culture conditions might explain some of these irregularities, especially as it appeared that tobacco does not rank very high as a suscept.

An experiment was carried out to determine the effects of age and the vigor of growth of the plant on the expression of primary acute (local necrotic lesions and ring spots) and secondary acute symptoms (systemic necrotic lesions, ring spots, and oak-leaf patterns). Three types of tobacco were used. Two ages and three ages were grown in three different sizes of soil containers as indicated in table 1.

Fertile composted soil was used throughout. The plants were grown in a greenhouse shaded with whitewash during the late summer. All plants were inoculated at the same time (Aug. 15) with nondiluted virus extract from heavily infected leaves of Scotia bean plants. Each tobacco plant was inoculated by wiping 4 top leaves, the topmost one ranging from 65 to 90 mm. long. There were 4 plants in each test group. At the time of inoculation the plants growing in the 6-inch clay pots were definitely pot-bound, those in the 8-inch clay pots were showing slight effects from potbinding, whereas plants growing in the boxes were almost as vigorous as those cultured in the field. The final data were recorded Sept. 6 when some of the plants had started to flower. The results are given in table 1.

TABLE 1.—*Data showing the influence of age and plant vigor on the expression of primary and secondary acute symptoms caused by the ring-spot virus in tobacco*

Collection or variety of plant used	Age of plants in days	Type of soil container used, the number of primary lesions per 100 cm.² of leaf surface, the percentage of plants with secondary acute symptoms and the degree of expression								
		6-inch pots			8-inch pots			Boxes[a]		
		Primary lesions	Secondary acute symptoms		Primary lesions	Secondary acute symptoms		Primary lesions	Secondary acute symptoms	
			Per cent of plants	Degree of expression		Per cent of plants	Degree of expression		Per cent of plants	Degree of expression
		Number			*Number*			*Number*		
T.I. 706	78	0	0	.....	0	0	.....	5.9	50	Mild
"	92	0	0	.....	0	0	.....	2.5	25	Trace
Samsun (Turkish)	78	0	0	.....	0.65	25	Mild	12.2	100	Very severe
	92	0	0	.....	1.30	25	Trace	5.5	100	Severe
	102	0	0	.....	0.57	0	.....	7.3	100	Severe
T.I. 448A	75	0	0	.....	4.56	25	Mild	29.3	100	Severe
	88	0	0	.....	0.81	25	Mild	11.7	100	Severe
	102	0	0	.....	0.73	25	Trace	12.53	50	Trace

[a] These boxes contained 1¼ cu. feet of soil per plant.

The data show that the pot-bound (retarded) plants were most resistant to the expression of acute symptoms. In all cases the most vigorous plants (cultured in boxes) showed the most severe symptoms. In no instance were the oldest plants the most susceptible. These results have been verified many times in numerous tests bearing on other phases of the problem.

Nonthrifty and older plants that manifested no signs of primary necrotic lesions sometimes did manifest obscure signs of local infection on the wiped leaves. Examination of such leaves by diffuse transmitted light, especially in a darkened room, revealed occasional light-green circular zones as illustrated in figure 1.

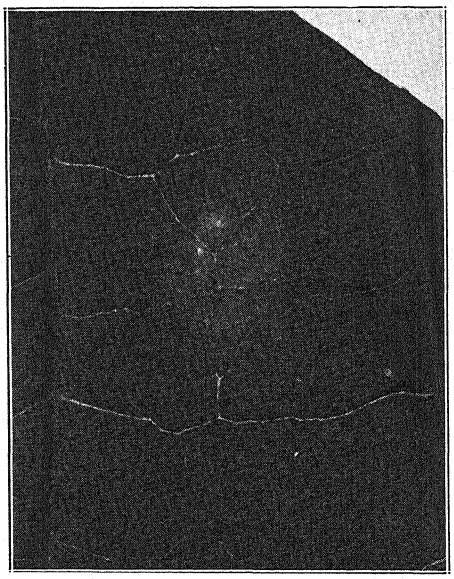


FIG. 1. Portion of leaf of Samsun tobacco illustrating one of the occasional primary light-green zones occurring when leaves on pot-bound plants are wiped with tobacco ring-spot virus. Photographed by transmitted and reflected light. ×2.

In table 1 it will be observed that T.I. 706 was more resistant than T.I. 448A and Samsun. Many tests have shown this resistance in T.I. 706. The difference in resistance between T.I. 448A and Samsun has been less consistent, but from observations made over a period of time, it appears that T.I. 448A is the more susceptible. This is an especially desirable tobacco for the ring-spot studies because it is highly resistant to the common-mosaic virus and its mutants (2, 3). The chlorotic patching, which Valleau (8) reported, has not been observed in the chronic diseased leaves of Samsun tobacco plants grown in these tests.

Samsun and T.I. 706 are highly susceptible to the common-mosaic virus and its mutants, in that they sustain a very high level of virus synthesis. Both tobaccos are highly tolerant, however, especially in the chronic phase and they complete their life cycles under ordinary culture conditions, in

spite of reductions in the chlorophyll. The secondary acute symptoms induced by the ring-spot virus in tobacco are frequently similar to those induced by alfalfa-mosaic virus 1B (10).

When young tobacco plants are inoculated with sufficiently active virus, the acute phase of the ring-spot disease tends to involve but 1 to 4 consecutive leaves above the point of inoculation and the onset of the chronic phase is hastened and, therefore, this phase involves the great majority of the leaves of the mature plant. On the other hand, when older vigorous plants are inoculated, the number of leaves on the mature plant that show the acute and chronic phases may be about equally divided or the acute phase may continue on all the leaves until the plant matures without the chronic phase

TABLE 2.—*The influence of age of tobacco plants inoculated with ring-spot virus, on the number of leaves in the secondary acute phase and in the chronic phase of the ring-spot disease in T.I. 448A tobacco. Inoculation was by the wiping method and plants were cultured in adjacent plats in the field.... The same lot of inoculum was used throughout*

Plant No.	Leaves with indicated symptoms and "skip" leaves[2] in plants inoculated when					
	42 days old			63 days old		
	Secondary acute symptoms	"Skips"	Chronic symptoms	Secondary acute symptoms	"Skips"[a]	Chronic symptoms
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1	1	0	40	35	6	0
2	4	0	38	36	4	0
3	3	0	41	13	6	20
4	2	0	42	19	1	19
5	5	0	36	16	1	22
6	1	0	43	37	0	0
7	3	0	44	24	4	11
8	4	0	38	19	1	11
9	4	0	39	11	0	30
10	3	0	40	33	7	0

[a] See table 4. In this test most of the "skips" were near the zone of inoculation. Leaves below the inoculated leaves were disregarded, as they did not show symptoms.

becoming evident. In either case, however, when the older plants are inoculated, there may be a number of "skips"[2] among the leaves that show the acute symptoms. Representative data in table 2 illustrate this.

The differences in reaction of young and old plants to the virus seems to be accounted for on the basis of resistance that increases with age, as it appears that virus invades the very young tissues and possibly the growing point most readily in young plants.

Effect of Inoculation Methods on the Type of Symptom Expression

Many inoculation tests made by the wiping method have shown that fine carborundum powder (600 grain) materially increases the expression of the primary and the secondary acute symptoms. A typical experiment is cited.

[2] "Skips" are leaves free of acute symptoms but flanked by leaves with acute symptoms.

On August 21, 1941, 55 vigorous healthy plants of T.I. 706 and 6 plants of Samsun, all from 8 to 10 inches tall, growing in a field plat, were inoculated. The virus extract was obtained from freshly collected leaves of T.I. 448A tobacco, all of which had severe acute symptoms of the ring-spot disease. They were clipped and pressed under hydraulic pressure and the extract was used without dilution. Alternate plants in each row of 8 to 10 plants were inoculated without carborundum by wiping the virus extract

TABLE 3.—*Increased infection with the ring-spot virus when carborundum powder was dusted on leaves before wiping with virus extract. Samsun (Turkish) tobacco plants used. The degree of the acute reaction is given for each leaf above the inoculated leaves on each plant to illustrate the irregularities leaf by leaf, especially when the amount of initial virus entering the plant is small. Data were taken at maturity of plants*

Leaves numbered upward from above those inoculated	Degree of secondary acute reaction in each leaf[a] inoculated					
	Without carborundum Plant No.			With carborundum Plant No.		
	1	3	5	2	4	6
25	.....	.....	.....	.....	.....	75
24	.....	0	.....	.....	.....	100
23	.....	0	.....	.....	0	90
22	.....	0	.....	100	25	100
21	.....	0	.....	100	100	100
20	0	0	0	100	75	90
19	0	0	0	100	100	100
18	0	0	0	100	100	100
17	0	0	0	100	90	85
16	80	0	80	100	100	100
15	0	0	0	100	40	30
14	1	0	1	100	100	5
13	5	0	5	100	90	100
12	0	5	0	100	95	25
11	10	0	10	100	100	40
10	0	0	0	100	95	30
9	0	0	0	75	95	1
8	40	0	40	75	60	60
7	0	0	0	40	90	1
6	0	0	0	30	50	1
5	0	0	0	50	5	10
4	0	0	0	20	75	3
3	0	0	0	30	5	1
2	0	0	0	5	0	0
1	0	0	0	25	0	0

[a] The plants inoculated without carborundum and with carborundum developed 7 and 46 primary lesions per plant, respectively. The values in the columns are percentages of severity based on a rating of 100 for a leaf with the most severe reaction as illustrated in figure 2, B.

on 4 top leaves of each plant, which leaves ranged from 4 to 12 inches long. The wiped leaves were rinsed with water. The remaining plants in the row were then inoculated by the carborundum-wipe method and rinsed with water. Thus, except for the carborundum, the inoculation procedure and the inoculum were the same in both sets of plants. Leaves of the same size, within narrow limits, and in the same position, were inoculated throughout.

The advantage gained from carborundum powder is clear from the summarized data in table 3. Although fresh nondiluted virus extract was

used, the number of primary lesions was exceedingly small. This may be explained in part by cool weather conditions that prevailed for a few days after inoculation. Data from the T.I. 706 tobacco plants are not presented, but they were consistent with those obtained from the Samsun plants.

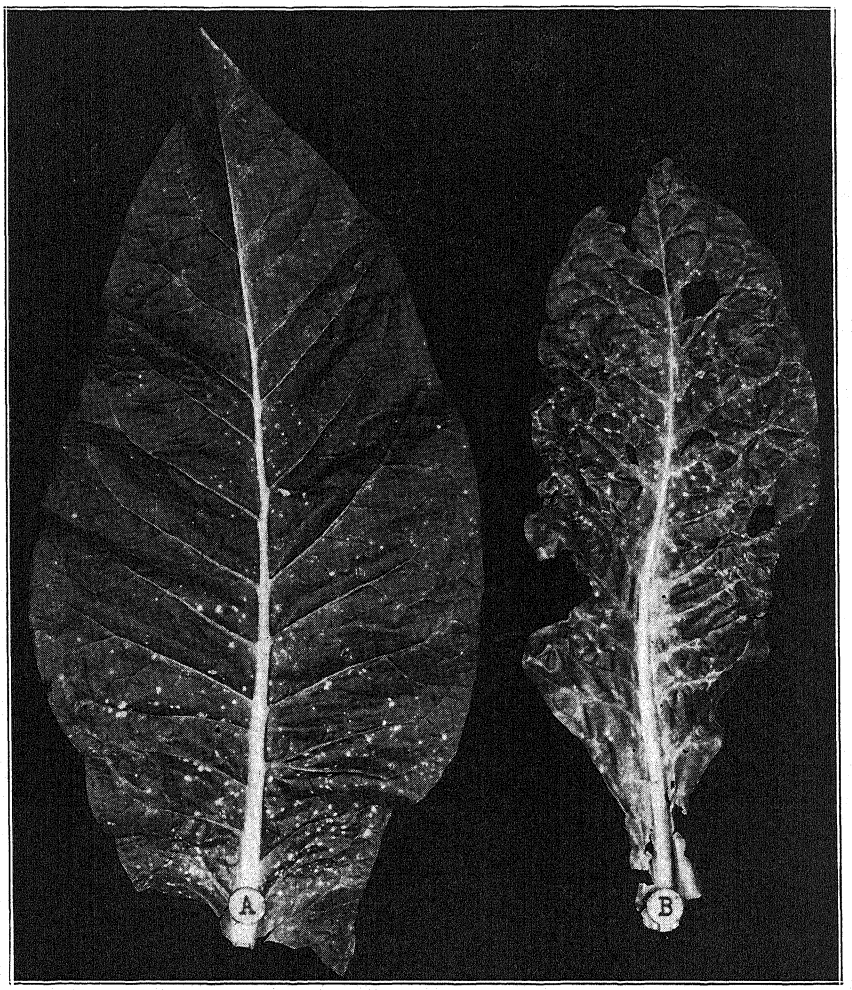


FIG. 2. Leaves of T.I. 448A tobacco showing weak (A) and severe (B) secondary acute symptoms of the ring-spot disease resulting from inoculations with extracts of low and high virus activity, respectively. These leaves were tenth above the inoculated leaves. $\times \frac{1}{2}$.

In table 3 the data for each leaf on 6 Samsun tobacco plants are presented to show the irregularity that occurs in the expression of secondary acute symptoms. These data illustrate the "skip" leaves referred to in table 2. Weak expression of acute symptoms and the number of symptom-free leaves has been associated with inoculation methods that favor the entry of small quantities of virus, and also with old plants and reduced vigor. Weak and severe secondary acute symptoms are illustrated in figure 2.

Another inoculation test was undertaken with a view to getting the greatest possible quantity of the ring-spot virus into the stem tip. In the resistance studies with T.I. 448A against the common-mosaic virus (3) and its yellow-mosaic mutants, it was found that "skip" leaves occurred. Frequently it was found that virus-free leaves were flanked by leaves carrying virus. Experience gained from the study of mosaic-resistant tobacco and related species leads to the belief that "skip" leaves can be regarded as good evidence of certain types of resistance in host plants when optimum culture conditions prevail.

All of the circumstantial evidence indicates that tobacco does not support a very high level of synthesis for the ring-spot virus, and, as a consequence, small amounts of the virus introduced into the plant do not incite a huge production of virus that quickly invades the plant. In order to counteract the natural resistance of the plant—within limits—it is necessary to introduce large amounts of virus in the inoculum.

Observations on the behavior of the ring-spot disease and preliminary assays for virus activity have supported Valleau's (8) view that the expression of the chronic phase probably depends on infection in or very near the primordial meristem. Accordingly, tests were conducted by methods that seemed most likely to force adequate amounts of virus into this region, and thereby favor the early onset of the chronic phase. Since plants of advanced age resist the chronic phase to a greater degree than young plants (table 2), tests were conducted with vigorous healthy plants 56 days old from the time of seeding, to determine if the appearance of the chronic phase can be hastened when the virus is introduced directly into the tip of the stem by means of a fine dissecting needle. Fifteen plants of T.I. 448A were inoculated in this manner with fresh active virus extract from cucumber and a like number of plants were inoculated by wiping the same virus extract on one leaf, from 75 mm. to 105 mm. long, at the top of each plant. Culture was in 6-inch earthen pots in a greenhouse.

In the series inoculated in the stem tips, 3 plants failed to show symptoms, 12 developed acute symptoms on 8 to 11 leaves per plant, and in 10 of these the acute symptoms were very severe. At the end of a 40-day period all 12 infected plants had passed well into the chronic phase. On the basis of leaf counts made in the dissected tips of plants in a similar stage of development as those inoculated, it appears likely that all, or nearly all of the leaves with acute symptoms were formed at the time of inoculation in the series inoculated in the stem tip.

In the series inoculated by wiping, all plants developed acute symptoms; in 13 plants the symptoms became severe, and in 2 plants they were weak. At the end of the 40-day period, the chronic phase had not appeared in any of these plants. The tip inoculation method was used in several tests and found to be erratic. Acute symptoms either failed to appear or they were very mild. Subsequent tests have indicated that the virus should be introduced very close to the growing point.

Since large quantities of virus influence the expression of acute symptoms so greatly (table 3), it was decided to amputate some of the apical leaves in aging plants and then inoculate leaves directly below the amputated zone with very active virus extracts. It seemed reasonable to believe that when the virus was deprived of access to several leaves, part of the large quantity of virus present in the wiped leaves would get into the apical zone sooner than if the larger apical leaves were left intact.

Leaves 3 to 4 mm. long and smaller were allowed to remain on the stem tip and the 5 or 6 leaves below these, which were from 10 to 60 mm. long, were carefully removed. This procedure was carried out on alternate plants in the rows in the field, the remaining plants serving as controls. In all, 70

TABLE 4.—*Data showing the influence of the amount of virus entering the wiped leaves, and also the influence of amputation of 5 or 6 small leaves near stem tips of inoculated plants on the expression of the acute and the chronic symptoms of the ring-spot disease in tobacco. No. T.I. 448A cultured in the field*

Items	Virus from cucumber leaves		Virus from tobacco leaves	
	No leaves amputated	5 or 6 leaves amputated	No leaves amputated	5 or 6 leaves amputated
Plants inoculated (number)	19	18	15	15
Primary lesions per cm.^{2} of leaf surface inoculated (number)	1	1	00.02	00.02
Plants with secondary acute symptoms (number)	19	18	5.0	14.0
Plants manifesting the chronic phase (number)	6	17	0	0
Leaves per plant above amputated leaves (average number)[a]	24.21	29.16	.....	.....
Leaves occurring in the acute phase[b] (per cent)	90.66	55.41	.....	.....
"Skip" leaves appearing in acute phase[c] (per cent)	6.70	00.00	.....	.....
Leaves appearing in the chronic phase (per cent)	9.33	44.58	.....	.....

[a] In the nonamputation control, the leaves corresponding to those amputated are not included in the counts. Leaves on main floral axis are included.

[b] The "skip" leaves were included.

[c] This percentage is based on the number of leaves in the acute phase, but the other percentages are based on the number of leaves above the zone of leaf amputation.

vigorous plants of T.I. 448A, 63 days old from seeding, were used. Inoculation was by the carborundum-wiping method. Four leaves, from 8.0 to 25.0 cm. long, and directly below the amputated leaves, and equivalent leaves on the controls, were inoculated and then rinsed with water. Fresh concentrated virus extract from infected cucumber leaves was diluted in 3 volumes of M/100 phosphate buffer at pH 7.0 (6) and used to inoculate 40 of the plants, and fresh concentrated virus extract from Samsun tobacco leaves in the chronic phase and diluted in 1 part of buffered solution, was used to inoculate the remaining 30 plants.

The results of this test are presented in table 4. The activity of the virus from cucumber was much greater than that of the virus from tobacco.

This is reflected in the number of local infection cites (primary lesions) and in the expression of the secondary acute symptoms.

Where the more active inoculum was used, primary lesions appeared 2 days after inoculation and in the nonamputated controls, the secondary acute symptoms appeared 6 days after inoculation in the leaves that corresponded to those removed in the amputation series. In the amputation series, the secondary acute symptoms appeared in the new apical leaves 10 days after inoculations, and within 2 days the symptoms on these leaves were the most severe ever observed by the writers. For a period of 10 days the secondary acute symptoms were clearly more severe in the amputation

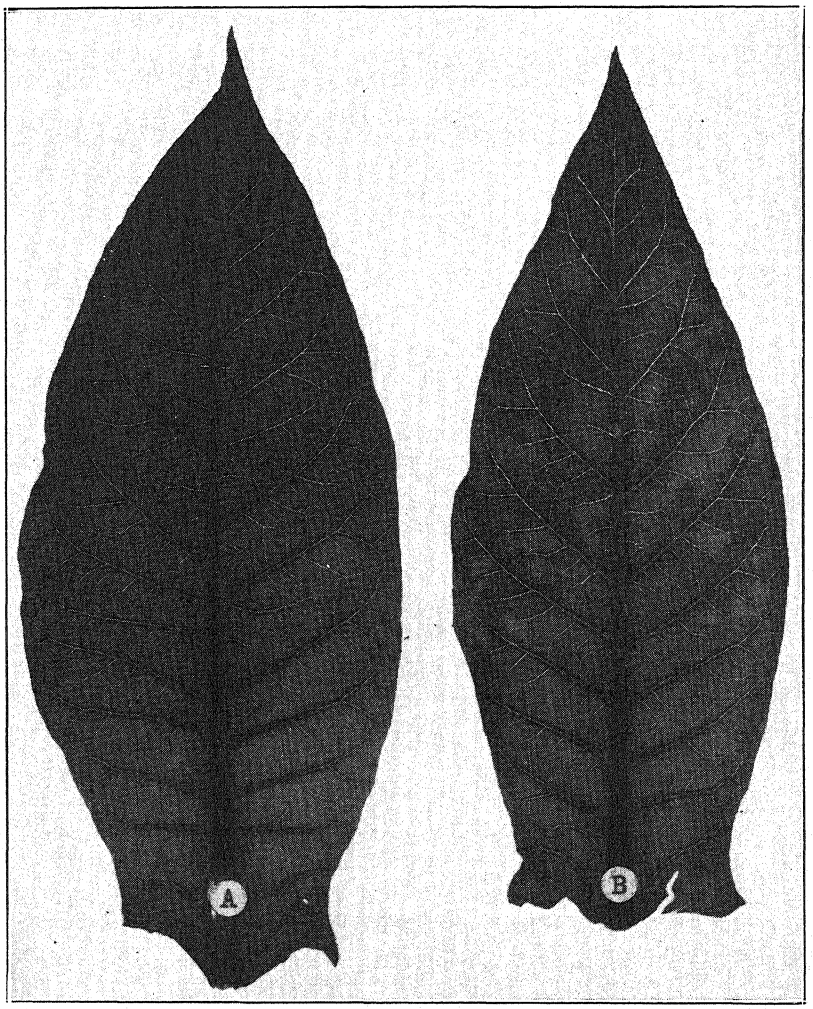


FIG. 3. Leaves of T.I. 448A tobacco, A, healthy and B, with mosaic mottling in the chronic stage of the ring-spot disease developed during cool periods. Photographed with transmitted and reflected light. $\times \frac{1}{2}$.

series than in the control. As the plants developed, the severity of the acute symptoms in the amputation series tended to become less in the new leaves, but there were few new leaves with reduced symptoms, as the chronic phase started to appear 10 days later (20 days after inoculation). During periods of cool weather, chlorotic patches and mosaic mottling appeared in leaves 10 to 20 cm. long on the plants that manifested the chronic phase. The chlorotic patches resembled those illustrated by Valleau (8), and the type of patching or heavy mottling that is characteristic of tobacco mild dark-green mosaic. A rather typical mosaic mottling is illustrated in figure 3, B. A distinct mosaic virus could not be isolated from these and similar leaves. Throughout the work it was observed that in plants of T.I. 448A (tobacco) grown in the field, the chlorotic patching and mosaic mottling tended to be most prevalent in the first 4 to 8 leaves with chronic symptoms. In table 4 it will be noted that no "skip" leaves appeared in the amputated series.

Where the less active virus from tobacco was used, the secondary acute symptoms were delayed, appearing 20 days after inoculation. The symptoms were very weak in the lower leaves, but they tended to increase progressively in the new leaves until they were moderately severe in the upper portion of the plants. Chronic symptoms failed to appear in any of the plants, but the data in table 4 show that amputation of the small leaves favored secondary acute symptoms.

In the amputation series inoculated with the highly active virus from cucumber, it is reasonably certain, from gross dissections made on similar plants, that more than half of the leaves that developed acute secondary symptoms were differentiated by the time virus entered the apical zone. Microscopic studies might have revealed a greater number of differentiated leaves.

This test indicates that the proximity of large quantities of the virus to the stem apex favors the expression of the chronic phase of the ring-spot disease. The small leaves directly above the amputated leaves developed the most severe acute symptoms ever noted by the writers, thus a large reservoir of virus was established close to the growing point, making it possible for the virus to surmount some form of natural resistance and enter very young leaves in quantity. The results of this test and the results obtained from the needle inoculations made directly into the stem tips, are in general agreement.

Although it is not the purpose of the present study to determine the relative quantities of ring-spot virus in different tissues, certain preliminary tests have been made, and the results are summarized.

"Skip" leaves varied with respect to the presence of detectable virus. Some contained detectable virus, whereas others did not. In leaves manifesting mild and moderate secondary acute symptoms, most of the normal green tissue is at first free of detectable virus, but later on virus enters some of these areas. In a general way, this resistance in leaves with the secondary acute symptoms of the ring-spot disease is suggestive of the resis-

tance shown in leaves of tobacco T.I. 448A against common-mosaic virus (wild type) and its yellow-mosaic mutants (3). However, the level of resistance is higher against the mosaic viruses than it is against the ring-spot virus.

Tobacco plants with the mild acute symptoms of ring spot apparently have no detectable virus in the stem apex; in plants with the severe acute symptoms, virus was detected in the stem apex, but at much lower concentration than in the ring-spot tissue of the leaves. In plants in the chronic stage, virus-activity tests were made on the stem tips[3] and on a series of leaves dissected from the tip. The virus activity tended to be slightly greater in the stem tips than in the leaves. The onset of the chronic phase may be determined by a relatively high level of virus activity in the stem tip rather than by mere presence of virus in that region of the stem.

Leaves 24 to 40 cm. long and produced during the chronic stage were sampled by means of cork borers. Each small disc was assayed separately on 2 to 10 cucumber seedlings. Thirty-one discs 11 to 19 mm. in diameter from three leaves were tested and virus was present in each. Sixty-six discs 4.5 mm. in diameter were taken from three other nearly mature leaves and tested. All but 5 of these contained virus. These results indicate that invasion is almost solid in the leaves produced during the chronic phase, yet the resistance of this tissue is so high that visible signs of disease are absent at temperatures that favor symptoms in a more susceptible host, such as cucumber, and in tobacco when infected with common mosaic virus.

Owing to a very large experimental error in the methods used for assaying the ring-spot virus, further study is necessary before critical interpretations are possible from assay data. Studies on the apical stem tissues are very difficult in Samsun and T.I. 448A tobacco. Preliminary work indicates that Maryland Mammoth tobacco offers possibilities for more accurate studies because of the unusually large size of the apical zone, and on account of its indeterminate vegetative growth when cultured in a long photoperiod.

Weight of Tobacco Leaves and Plants as Influenced by the Ring-spot Disease

During the season of 1941, 36 plants of T.I. 448A tobacco were grown in the field in three rows of 12 plants each. Four plants in each row were used as noninoculated controls and the other 24 plants were inoculated. The soil was uniform, and well adapted for commercial tobacco culture, and the plat was practically level.

On July 3 when the plants were 25.0 to 35.0 cm. tall, they were inoculated by wiping virus extract on 4 top leaves, 8.0 to 25.0 cm. long. No carborundum was used. The inoculum consisted of an extract from infected primary leaves of Scotia beans diluted in 4 parts of phosphate buffered solution.

The controls remained free of ring spot and other diseases. The inoculated leaves developed primary lesions at the rate of about 1 lesion per

[3] Stem tips included all differentiated leaves up to 20 mm. long.

square centimeter of surface. All plants developed severe secondary acute symptoms, 5 plants failed to show the chronic phase, whereas in the remaining 19 plants, the chronic phase appeared in from 11 to 31 top leaves.

Owing to the severity of the acute reaction, it was not possible to obtain the information desired on the chronic phase. However, the yield data do show the marked reduction caused by the acute symptoms.

Leaves were harvested at three periods during the season, beginning above those inoculated on each plant, when the leaves had attained their full development. Thirty market-type leaves were obtained from each plant in the control and in the inoculated series.

At each harvest the yield (green weight) from the controls was almost double that from the diseased plants. The average total yield in the controls was 1.004 kilograms per plant, and in the infected series it was 0.502 kilograms per plant.

During the season of 1942, 30 plants of T.I. 448A tobacco, grown in the field, were prepared for a yield test on the chronic phase of the disease, but the inoculum used proved to be too weak. All the plants developed primary lesions, but only 24 developed weak secondary acute symptoms, one of which developed the chronic stage late. Six plants gave no signs of systemic infection. Rather than lose the entire season, another test with 30 plants of the same variety of tobacco, similarly grown, was prepared as soon as it became evident that a weak inoculum had been used. Fifteen of these plants in the second series were inoculated. As they were very young when inoculated, they developed the chronic phase quickly. The acute phase involved only 1 to 4 of the lower leaves per plant.

During the season it was noted that the infected plants were not so robust as the controls. As the season advanced, there was some irregularity in the vigor of all the plants, but the controls averaged the more robust. Just before flowering, the plants were harvested and each plant, stem and all leaves, was weighed.

The healthy controls gave an average green weight per plant of 1.483 kilograms, whereas the infected plants gave an average weight per plant of 1.034 kilograms. Although the infected plants manifested no acute symptoms after they were transplanted to the field, they showed a 30.28 per cent reduction in yield.

RESULTS WITH CUCUMBER

A limited survey of varieties of cucumber (*Cucumeris sativus* L.) indicated that young plants of the variety Early White Spine are very susceptible to the tobacco ring-spot virus. When inoculated with nondiluted native virus and cultured at warm temperatures, young plants are usually killed. It was found that mosaic mottling (Fig. 4) occurred in the chronic phase more regularly and at somewhat higher temperatures than in tobacco. This mosaic is readily confused with the mosaics induced by the cucumber-mosaic viruses, and with alfalfa mosaic virus 1A in cucumber (10).

During the summer of 1942, ten Early White Spine cucumber plants were grown in rich soil in large, deep, ground beds in a greenhouse without shade. Five of these plants were inoculated when they were small with tobacco ring-spot virus and the others left as controls.

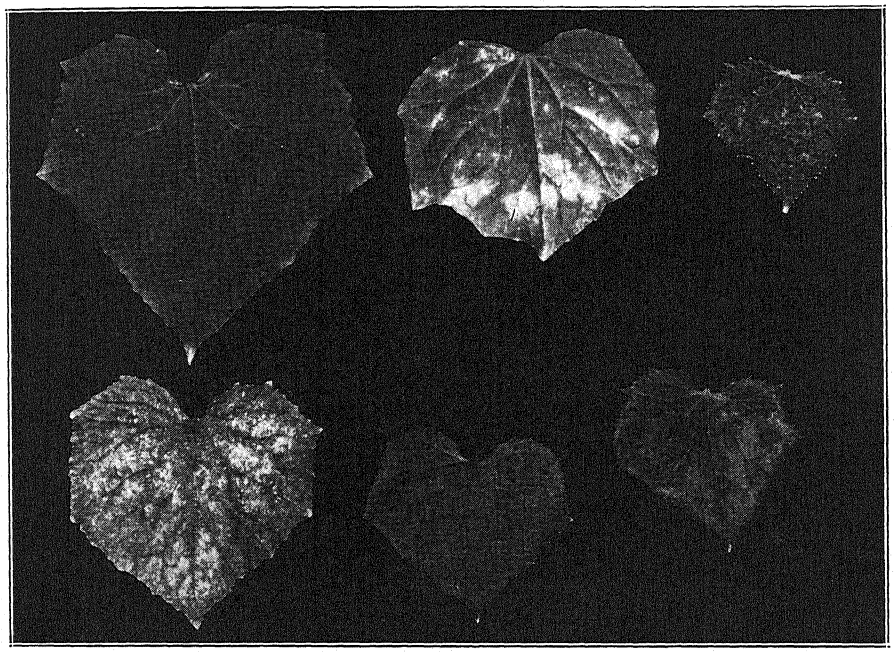


FIG. 4. Leaves of Early White Spine cucumber. Healthy leaf upper left. Leaf at right of healthy leaf has primary lesions, other leaves show various expressions of secondary chlorosis and mosaic mottling during the chronic phase of the ring-spot virus infection. $\times \frac{1}{2}$.

All inoculated plants developed severe acute symptoms, and mosaic appeared at intervals on the new leaves throughout the season (Fig. 4). The control plants remained free from all diseases until at the end of the test when powdery mildew appeared. Throughout the season, the control plants were much more robust than the inoculated ones, and the latter gave no signs of improved vigor as they developed. Fruit was removed from the vines at intervals throughout the season, and at the end of the season the vines only were weighed.

TABLE 5.—*Data showing reduced yield caused by the tobacco ring-spot virus in Early White Spine cucumber plants grown in ground beds of fertile soil, in a greenhouse without shade, during the summer months, 1942*

Items	Healthy	Diseased
Average weight per plant without fruit	8.63 kg.	0.288 kg.
Average width of largest leaf per plant	22.13 cm.	13.34 cm.
Average length of longest petiole per plant	29.50 cm.	7.90 cm.
Average diameter of longest petiole per plant	8.33 mm.	5.50 mm.
Average length of longest runner per plant	3.45 m.	1.62 m.

The results of this test are given in table 5 and show that the ring-spot virus reduced the green weight of the plants about 97 per cent. This reduced weight was reflected in the smaller size of the leaves and in shorter length and smaller diameter of the runners.

RESULTS ON BEAN

In *Phaseolus vulgaris* L., var. Scotia, the ring-spot virus induced local lesions on the wiped primary leaves. At temperatures near 33° C., systemic infection was more rapid than at 22.5° C., and acute necrosis involved the secondary leaves, stem, and entire plant, causing death.

RESULTS ON PANSY

Pansy plants (*Viola tricolor,* var. Swiss Giant) were inoculated by all the methods used with tobacco and cucumber, and very active virus extracts were used as inoculum. The virus entered the plant readily when the leaves were wiped, but no signs of infection appeared on the wiped leaves. Occasionally vein clearing appeared in the new leaves, but no other signs appeared. The virus remained in the infected plants as long as they could be kept alive in the greenhouse. These tests have been repeated many times and always the same results were obtained. These results are at variance with those reported by Wingard (9).

DISCUSSION

The ring-spot disease and the yellow mosaic disease (5) in Samsun (Turkish) tobacco—each manifest an acute stage with pronounced symptoms and a chronic stage with reduced symptoms. Leaves well differentiated at the time of invasion show acute symptoms while leaves that are invaded at or near the time of their differentiation show chronic symptoms. However, the two diseases represent different levels of disease expressions. In the case of the yellow-mosaic disease the level of plant resistance was so low that in the chronic phase the reduced disease reactions were still very marked, whereas, with the ring-spot disease the level of resistance was so high in Samsun tobacco that ring-spot and mottling were not observed in the chronic diseased leaves. This reduced severity of the ring-spot disease was reflected in other varieties of tobacco, including genotypes that are highly resistant to tobacco mosaic. However, in some of these varieties and genotypes chlorosis and mosaic mottling appeared in certain chronic ring-spot diseased leaves at moderately low culture temperatures.

In pansy, resistance was so great that typical ring-spot symptoms were not apparent in any form, even though virus invaded the plant. However, in Early White Spine cucumber, the level of resistance to the ring-spot virus was sufficiently low to enable the expression of mosaic symptoms throughout most of the chronic phase.

Resistance to the ring-spot disease in tobacco was evidenced further by the fact that large amounts of virus and ideal culture conditions were neces-

sary to insure good acute reactions, especially in plants beyond the juvenile stage; and in such plants, the chronic phase appeared irregularly or not at all unless measures were taken to force large quantities of the virus into the very young apical tissues. It seems apparent that the level of virus synthesis is relatively low and that virus enters the meristematic tissue with difficulty. However, when virus does get into this tissue in sufficient quantity, perhaps into the apical cell, the subsequent young leaves manifest surprisingly few areas that are free from detectable virus. Since it appears that the characteristic masked or modified reaction of these leaves to the virus is predetermined by a high level of natural resistance in the very young tissues at the time of invasion, we are not at liberty to refer to these leaves as having recovered from the disease.

When these chronic diseased leaves were wiped with active ring-spot virus, they did not produce the primary acute reactions (necrotic lesions the ring spots) that resulted when healthy thrifty leaves were wiped with virus. The same results were obtained when yellow-mosaic virus was wiped on the chronic yellow-mosaic leaves on plants from the cross (*Nicotiana tabacum* × *Nicotiana longiflora* × *N. tabacum* (5). To expect these inoculated chronic-diseased leaves to react in the same manner as do inoculated healthy thrifty leaves would be contrary to logical expectations based on the evidence set forth in the studies on yellow mosaic (5), and to refer to this phenomenon as acquired immunity merely confuses the issue.

The growth-phase phenomena in the vertebrates differ so greatly from those in the seed plants, that the drawing of analogies (7) from either is extremely hazardous. The individual leaves of a tobacco plant possibly are comparable in a way to individual animals, but even this analogy is rather "far fetched." The practice of regarding the suppression of apparent symptoms in chronic diseases as acquired immunity, seems to accomplish nothing and it tends to obscure the more tangible lines of attack on the problem of disease expression and natural resistance in relation to developmental changes in the individual and to the gene mechanism.

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LATENT VIRUS OF DODDER AND ITS EFFECT ON SUGAR BEET AND OTHER PLANTS

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INTRODUCTION

In the spring of 1938 dodder, *Cuscuta californica* Choisy, was collected from a number of plants of a desert shrub known as California buckwheat, *Eriogonum fasciculatum* Benth., near Riverside, California, and placed on healthy sugar beets in the greenhouse preliminary to use in transmission experiments with the virus of sugar beet curly top. Within 10 to 15 days after the dodder began to grow on its new host, the sugar beet plants began to develop a type of chlorotic spotting or mottling that resembled somewhat that produced by some phases of common beet mosaic. However, the chlorotic areas were smaller, more uniform, and more intensely yellow than those commonly produced by beet mosaic and the leaves of affected plants were deformed. In further tests it was found that *C. californica,* parasitizing a number of desert species, was infected by a virus capable of producing these symptoms.

The virus is so unusual in its manner of occurrence and in the type of symptoms produced on sugar beet that it seemed worthwhile to make a more detailed study of its characteristics and host range. As will be shown, these studies revealed that a wide range of economic plants is susceptible to infection but the disease with its peculiar symptomology that would enable recognition has not been found in the field on any of these susceptible economic plants.

SYMPTOMS AND HOST RANGE

The host range of this virus, under desert conditions, has not been studied extensively. The virus was recovered only from dodder, *Cuscuta californica,* on which it produced no recognized symptoms of disease. No evidence of a diseased condition was found on any of the host plants on which infected dodder was growing. No virus was obtained from California buckwheat, *Eriogonum fasciculatum,* that had been parasitized by infected *Cuscuta californnica* and additional evidence obtained under greenhouse conditions indicates that this common host plant of dodder is immune from the virus. No other desert host plants of dodder were tested for presence of virus.

Using dodder as the agent of transfer, the virus was transmitted to several crop plants and to a number of common weeds. The symptoms of disease varied considerably on different host plants, but, since a type of mottling was more or less typical on most of the known susceptible species, the disease may be classified as a mosaic. The name Dodder latent mosaic is suggested for the disease. Representative symptoms are described on the following plants.

Sugar Beet (*Beta vulgaris L.*). First symptoms appeared usually on young leaves and consisted of more or less circular yellowish spots of various sizes. Spots were separate or confluent and in some instances affected leaves were almost completely yellow. Leaves produced later had less yellow color and the chlorotic spots were more widely scattered and larger, producing a mottled condition. Typically diseased leaves in early stages of attack are shown in figure 1.

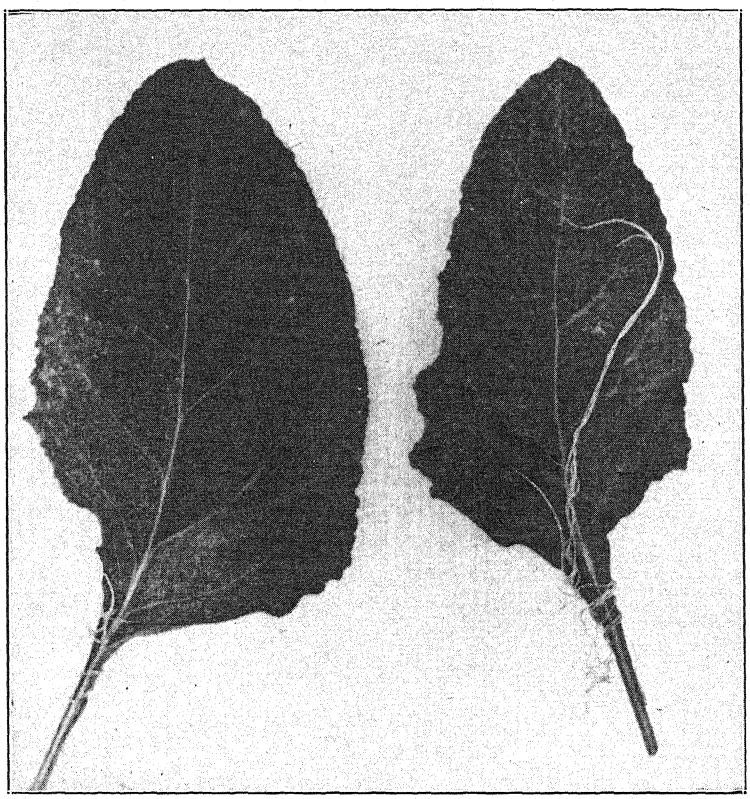


FIG. 1. Early symptoms of disease on leaves of sugar beet plants inoculated by means of *Cuscuta californica*.

Leaves with the more severe symptoms were somewhat dwarfed, sometimes crinkled with irregular margins. Usually, new growth of the affected plant ceased to show symptoms after 6 to 8 leaves were produced. In rare cases this recovery from symptoms was not complete and faded areas were observed on leaves produced several weeks after infection. In most cases, however, plants completely recovered and no symptoms were evident on subsequent growth and the virus did not appear to interfere seriously with further development under greenhouse conditions.

Cantaloupe (*Cucumis melo L., var. Rockyford*). Leaves 2 to 3 cm. in diameter usually were the first to show symptoms, although yellow spots

appeared first on older leaves in some instances. On the younger leaves, chlorotic spots 2 to 3 mm., or more, long and about 1 mm. wide were produced on the veins. In places the growth of the veins was markedly retarded, resulting in a curled and twisted condition of the affected leaf. Chlorosis and necrosis increased as the leaf matured, and at maturity the leaf was about half normal size. Sometimes such leaves died prematurely. Two to four leaves showed these characteristics, but the severity of the symptoms decreased as more leaves were produced until a chronic condition was reached in which leaves, although somewhat reduced in size, were more or less normal in shape. Spotting of the type shown in figure 2 continued

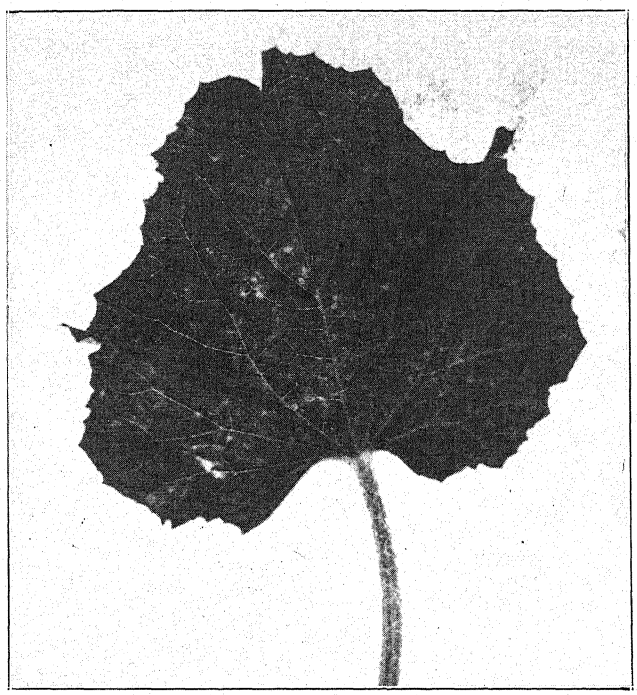


FIG. 2. Chronic symptoms of disease on cantaloupe, var. Rockyford.

to be produced. The chlorotic spots were small but rather numerous and more conspicuous after the leaf was more than half grown. As the leaves became older, the chlorotic spots sometimes became necrotic in the center.

Plants inoculated in the greenhouse and transplanted to the field grew slowly and produced vines only about two-thirds as long as those on non-inoculated check plants. Leaves were much smaller than on normal plants and the melons were small and of poor quality. This evidence indicates that the latent virus of dodder is capable of causing a serious disease of cantaloupe if an efficient vector be available.

Potato (Solanum tuberosum L.). On potato plants of the White Rose variety, circular, dark, necrotic lesions 1 to 3 mm. in diameter were formed

on half-grown leaves 10 to 15 days after inoculation. The necrotic spots were sparse on some leaves, but more often they were abundant and more or less confluent. Some of the leaflets on which necrotic spots were numerous died before attaining maturity. In some cases, especially in the early stages of attack, the disease was more severe on one side of the leaf than on the other, resulting in the curving of the midrib and in death of some or all of the leaflets on the more severely affected side (Fig. 3, A).

Recovery from symptoms began to take place after 6 to 12 diseased leaves had been produced. In the initial stages of recovery necrosis became less marked; leaves were smaller, and mottling of the type shown in figure 3, B, involving chlorotic areas of various sizes and shapes with ill-defined margins,

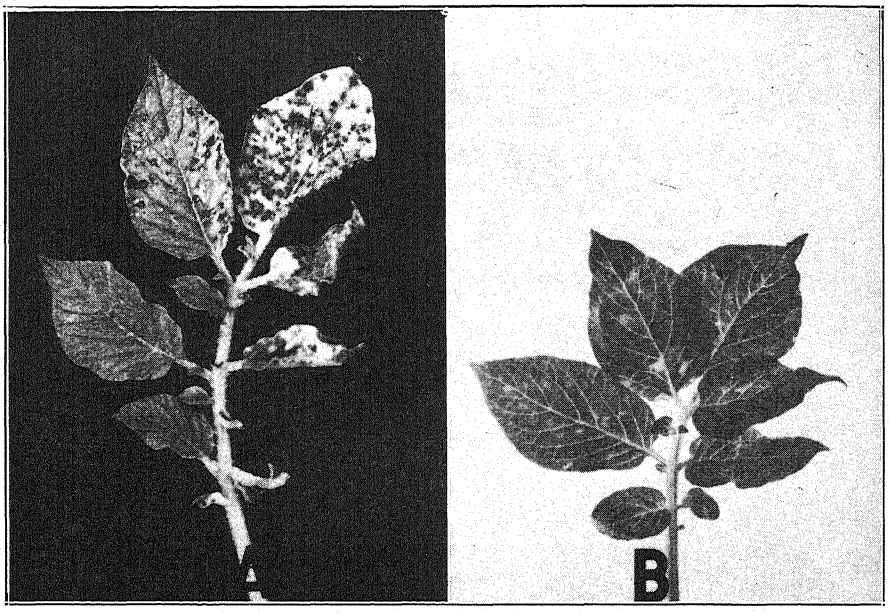


FIG. 3. Two phases of disease on leaves of potato, var. Early Rose. A, early stage of attack characterized by spotting, necrosis, and death of leaflets. B, later stage characterized by dwarfing and mottling.

was produced. As growth continued, mottling became less conspicuous until, after the production of 4 to 8 mottled leaves, growth again became normal.

Tomato (*Lycopersicon esculentum L.*). The younger leaves of tomato plants began to show water-soaked spots 7 to 14 days after inoculation. The spots turned brown within a few hours as the affected tissue dried. Spots were more or less circular and varied in size from 1 to 10 mm. in diameter. They were numerous and confluent or scarce. Usually not more than 3 leaves showed these necrotic spots. A very mild type of mottling was produced on the next 3 or 4 leaves. The terminal leaflets tended to twist, and often the lower surface was turned upward. Later growth appeared

normal. Diseased plants were not dwarfed either in the greenhouse or in the field and the yield of fruit seemed not to be decreased.

Celery (*Apium graveolens L.*). Very conspicuous primary symptoms were produced on the Golden Self Blanching variety of celery. The disease first made its appearance on leaves that were about one-third grown. In an area usually extending outward from the midrib into the leaflets, often chlorophyll was almost completely lacking and the affected parts were golden-yellow. The stage of development of the leaf when the virus entered evidently influenced extent and location of the chlorotic areas. Two to 4 leaves showed this type of chlorosis. The next 2 to 3 leaves were much

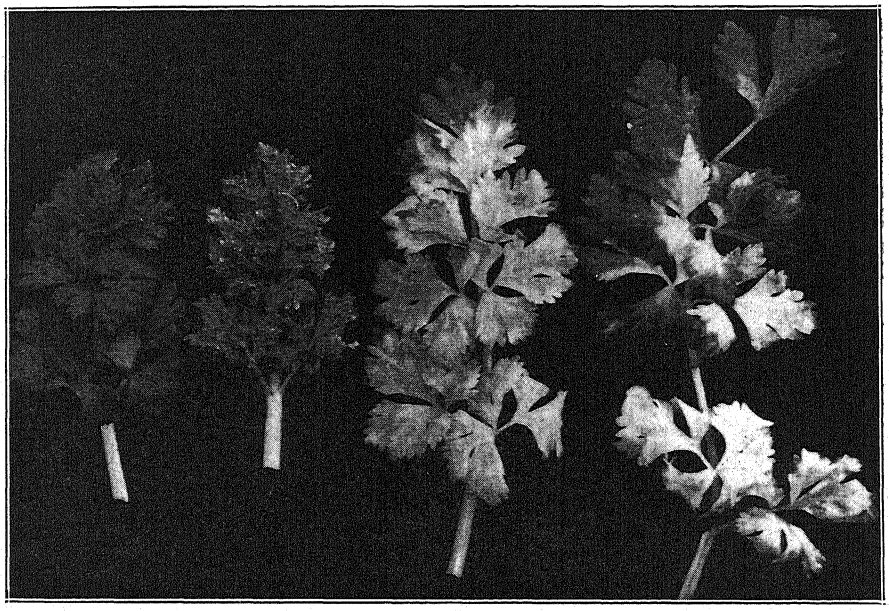


FIG. 4. Leaves of Golden Self Blanching celery showing (right to left) primary symptoms of disease and stages of recovery.

reduced in size and the leaflets were small, crinkled, and distinctly mottled. Following the production of these dwarfed and mottled leaves, recovery from symptoms was so complete that no evidence of abnormality was detected on any of the subsequent growth. A series of leaves of the Golden Self Blanching variety, showing transition from primary chlorosis through mottling to complete recovery, is shown in figure 4. Symptoms were somewhat less severe on the variety Utah.

Buckwheat (*Fagopyrum esculentum Moench*). Water-soaked spots, circular or more or less irregular in outline and varying in size, were produced on the young leaves of buckwheat plants 4 to 8 days after inoculation. These spots soon turned brown and the tissue became necrotic. This type of spot was restricted to 3 to 5 leaves. Later growth produced leaves somewhat reduced in size, mottled, and often rolled and deformed (Fig. 5). Under greenhouse conditions the yield of seeds was reduced considerably.

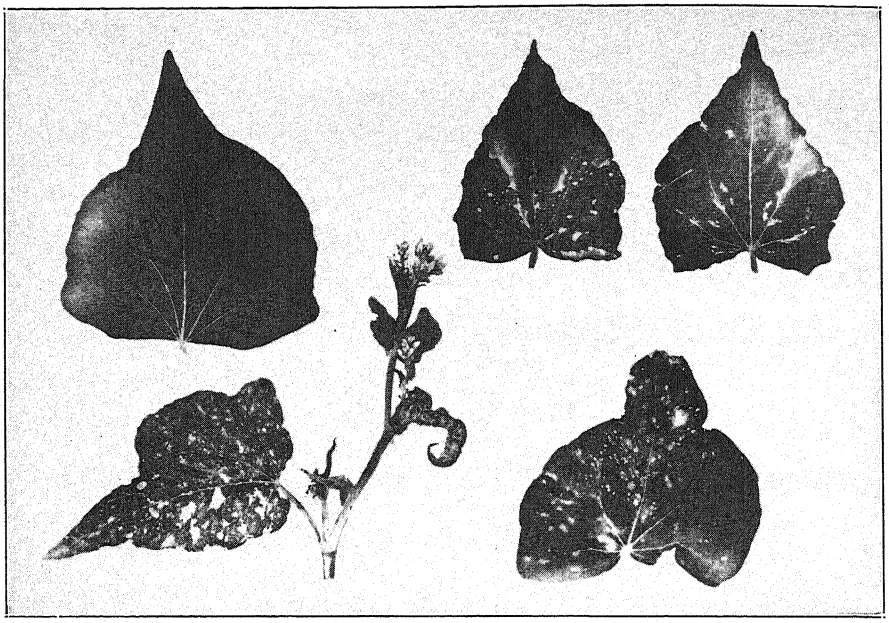


FIG. 5. Primary (separate leaves) and secondary (flowering shoot) symptoms on buckwheat.

Pokeweed (Phytolacca americana L.). Inoculations made by rubbing juice of infected plants over the surface of mature leaves of pokeweed resulted in the production of numerous local lesions usually on the third



FIG. 6. Typical symptoms of the disease on young pokeweed plants. The spots on the lower leaves are primary lesions produced by juice inoculation. Symptoms on the younger leaves are characteristic of those produced by systemic infection.

or fourth day following inoculation. The lesions appeared first as small, round, water-soaked areas about 1 mm. in diameter. They soon became necrotic and turned light-brown (Fig. 7, A). Subsequent development varied, depending on conditions. In most cases, as the leaves aged, reddish

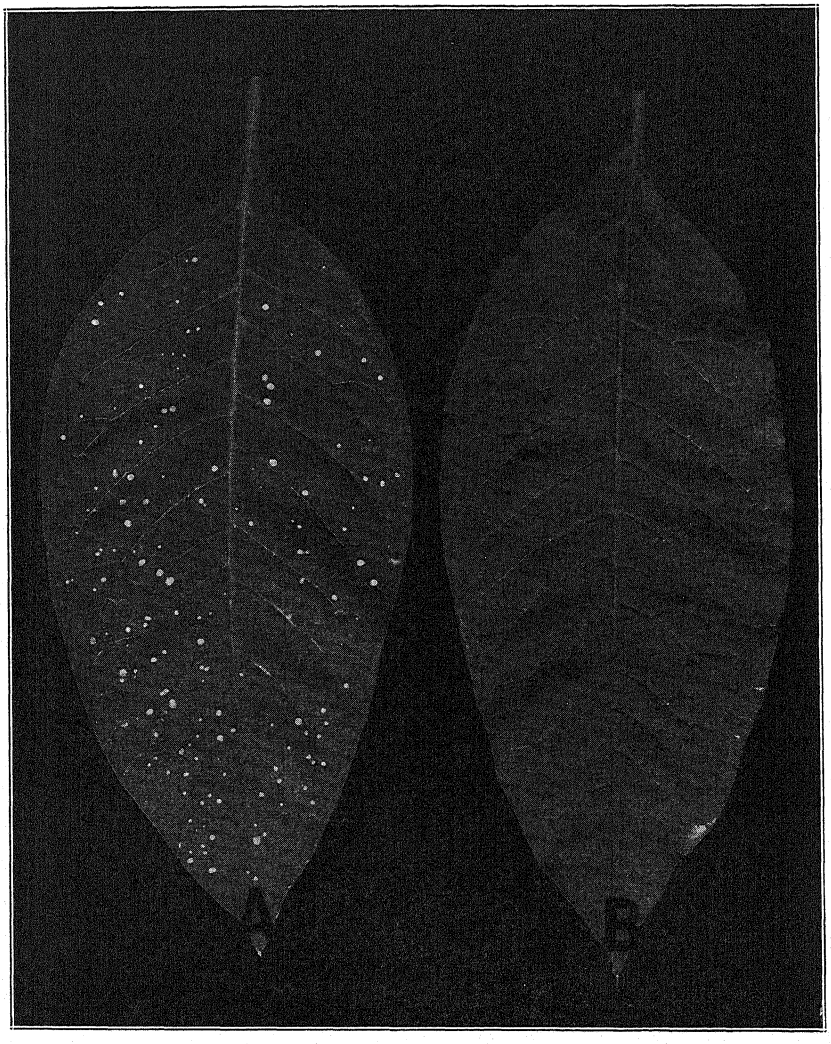


FIG. 7. Two leaves of approximately the same age from pokeweed inoculated by the rubbing technique, using juice from recently infected pokeweed. Leaf A from a previously noninoculated plant and leaf B from a plant that had ceased to produce symptoms.

rings were formed around the necrotic areas and outside of these, broader rings of faded tissue were noted. In some cases on older leaves the primary lesions were limited to faded circular spots with no evident necrosis.

The first evidence of systemic infection consisted usually of a wilting of the tip of one of the smaller leaves, followed within a few hours by the pro-

duction of roundish water-soaked lesions on this and other young leaves. The lesions soon turned brown. Often, one of the smaller leaves was killed outright. Usually, necrosis was produced on 3 to 5 leaves (Fig. 6), but the severity of the diseased condition decreased rapidly in subsequent growth. After the plant passed from the necrotic stage, 3 to 5 leaves were formed that showed mild vein translucency and a type of mottling. This type of symptom was followed by complete or almost complete recovery from symptoms. In rare cases, leaves on recovered plants were smaller than normal, obscurely faded in the interveinal areas, and the veins of young leaves were slightly translucent.

The leaves produced after the plants recovered were immune from production of primary lesions following inoculation by the rubbing technique. Figure 7, A and B, shows typical results obtained from inoculation of leaves of the same size and age on noninfected (7, A) and recovered (7, B) plants. Leaves such as that shown in figure 7, A, that had only primary lesions were not completely immune from further infection. Virus concentration in symptomless parts of diseased plants was very low compared to that in parts with severe symptoms, as indicated by the relative numbers of primary lesions produced on pokeweed leaves by inoculations with juice from the two types of tissue.

Other Susceptible Plants. Tests were made on a number of other plants to determine susceptibility. The following species were found susceptible and to produce necrosis of varying degrees of severity followed by recovery from symptoms: water pimpernel (*Samolus floribundus* N.B.K.), knotweed (*Polygonum pennsylvanicum* L.), common plantain (*Plantago major* L.), sowbane (*Chenopodium murale* L.) and lamb's quarters (*C. album* L.). *Nicotiana palmeri* Gray also is susceptible, but symptoms are limited to a type of vein clearing from which the plant soon recovers.

By use of virus-free dodder, *Cuscuta californica,* virus was recovered from the following species of plants on which no symptoms were observed: tree tobacco (*Nicotiana glauca* Graham), *Nicotiana rustica* L., vars. English, Iowa, *pumila,* and *jamaicensis,* tobacco (*Nicotiana tabacum* L.) var. Turkish, and mustard (*Brassica incana* (L.) F. W. Schultz).

Nonreactive and Immune Plants. No symptoms were noted on the following species of plants, but no attempts were made to recover virus: *Nicotiana sylvestris* Speg. and Comes, Jimson weed (*Datura stramonium* L.), Tolguacha (*D. meteloides* DC.), *Solanum integrifolium* Poir., *S. gilo* Radd., pigweed (*Amaranthus retroflexus* L.), tumble-weed (*A. graecizans* L.), chickweed (*Stellaria media* L.), hawkweed (*Hieracium argutum* Nutt.), *Sisymbrium irio* L., yellow sweet clover (*Melilotus indica* (L.) All.), squash (*Cucurbita maxima* Duchesne) var. Hubbard, pumpkin (*Cucurbita pepo* L.) var. Jack o'Lantern, bean (*Phaseolus vulgaris* L.) vars. Stringless Green Pod and Kentucky Wonder, garden pea (*Pisum sativum* L.) var. Little Gem, stock (*Matthiola incana* R. Br.), carrot (*Daucus carota* L.), and parsnip (*Pastinaca sativa* L.).

The following species of plants appear to be immune, since no virus was recovered from plants by juice inoculation to pokeweed or by use of virus-free dodder: California buckwheat (*Eriogonum fasciculatum*), sunflower (*Helianthus annuus* L.), cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.), belladona (*Atropa belladonna* L.) and mullein (*Verbascum thapsus* L.).

TRANSMISSION OF THE DISEASE

Transmission by Use of Dodder. Three species of dodder, *Cuscuta californica, C. subinclusa* Dur. and Hilg., and *C. campestris* Yuncker, when established on diseased plants, are able to pick up the virus of this disease. All 3 species of dodder retain the virus indefinitely when growing on plants immune from infection, and evidently are hosts of the virus. When infected dodder is trained to a susceptible plant, infection follows in all cases so far as known, after the dodder becomes established. The efficiency of these 3 species of dodder in the transmission of the virus has aided greatly in the study of the host range of the virus.

Tests of Insects. Since it seems improbable that the virus would be maintained indefinitely in nature through transmission by dodder alone, it is logical to postulate that transmission by insects takes place. Search for an insect vector under field conditions has been limited to a few localities. No insect that would appear promising as a vector has been located on dodder or on any of the plants on which dodder was growing.

Under greenhouse conditions there has been no evidence of spread of the disease in compartments where red spiders, thrips, mealy bugs, or the sugar beet-root aphids were present. Extensive transmission tests, using the peach aphid, *Myzus persicae* Sulz., certain unidentified species of aphids, and the beet leaf hopper, *Eutettix tenellus* (Baker), have given negative results.

Absence of the disease, so far as known, from crop plants indicates that if an insect is involved in its spread, this vector is closely limited in its feeding to desert species, and thus has little opportunity to carry the virus to the crop plants shown to be susceptible to infection.

Transmission by Juice Inoculation. Juice inoculations were made by gently rubbing the surface of leaves or stems, on which a small amount of abrasive had been sprinkled, with a cloth pad saturated with inoculum. Infection was produced on 4 species of plants; pokeweed, *Chenopodium murale,* sugar beet, and *Cuscuta campestris.* Of these plants, pokeweed is by far the most susceptible to infection. Frequently, as many as 200 primary lesions per leaf are produced on inoculated plants. On smaller plants evidence of systemic infection soon follows, but on plants approaching the blossoming stage, often no further symptoms are produced. *Chenopodium murale* appears also to be rather susceptible to infection, since 34 of 48 inoculated plants became diseased. Sugar beet is more resistant. Only 26 of 120 inoculated plants became infected, and the largest number infected

in a single test was 9 plants infected of 20 inoculated. Rapidly growing plants appeared to be more susceptible to infection than slow growing plants. *Cuscuta campestris* probably is still more resistant to infection. Of 40 seedling plants inoculated and placed on pokeweed, only one gave evidence of being infected. Of 20 large plants inoculated, 3 became infected.

In addition to the 4 species of plants already mentioned in connection with juice inoculation, all other species of plants found susceptible to infection through inoculation by use of dodder were tested for susceptibility to infection by juice inoculation. Extensive tests were made especially with celery, cantaloupe, tomato, and buckwheat, all of which show marked symptoms of disease when infected through dodder. No evidence of infection was observed on any of the juice-inoculated plants of these species nor on any of the plants of other species inoculated, with the exceptions already noted. However, juice from diseased plants of celery, cantaloupe, tomato, and buckwheat produced primary lesions on pokeweed, showing that the virus was not quickly inactivated in the extracted juice of these plants. In

TABLE 1.—*Results of tests of seeds of susceptible species of plants for transmission of virus*

Plants from which seeds were taken for tests for presence of virus	Seeds tested	Seeds found to carry virus
	Number	*Number*
Cantaloupe var. Rockyford	882	0
Pokeweed	1073	0
Buckwheat	121	0
Cuscuta californica	163	0
Cuscuta campestris	1080	52

view of this evidence it seems probable that failure to obtain infection through juice inoculation in a number of known hosts of the virus is due to reactions in the injured cells of inoculated leaves that prevent increase or transport of the virus.

Transmission Through Seeds. Seeds were collected from infected plants of Rockyford cantaloupe, pokeweed, buckwheat, *Cuscuta campestris,* and *C. californica* for tests to determine whether the virus is seed-transmissible. With the exception of *C. californica,* the seeds were collected from the diseased plants soon after they were mature and planted within a few days to reduce the possibility of inactivation of the virus in stored seeds.

The seeds of cantaloupe, buckwheat, and pokeweed were planted in pots and the plants were held until they attained considerable size before they were discarded. The seeds of the two species of dodder were allowed to germinate in flats and the seedlings were transferred to small plants of pokeweed or sugar beet to determine whether they transmitted the virus to these test plants. The results of all tests are shown in table 1.

All of the seedlings from seeds of diseased cantaloupe, buckwheat, and pokeweed were healthy in appearance. Since neither cantaloupe nor buck-

wheat completely recovers from symptoms of the disease, it may be assumed that seedlings would show symptoms if infected. However, since pokeweed recovers from symptoms, it is by no means certain that infected seedlings from seeds of diseased plants would show symptoms. It was found. however, when the seedlings were inoculated with juice from recently infected pokeweed, that primary lesions were produced on all plants from the seeds tested. Since recovered pokeweed plants are immune from the production of primary lesions, this result is considered proof that the seedlings were virus-free.

Although no evidence was obtained that virus was transmitted through the seeds of cantaloupe, buckwheat, and pokeweed, the evidence is conclusive that it was transmitted through the seeds of *Cuscuta campestris*. In the tests made, 4.8 per cent of the seeds from infected plants carried virus. The results from seeds of *C. californica* are inconclusive, since the number of seeds tested is small and the seeds were more than a year old at the time of test. Unfortunately, neither *C. californica* nor *C. subinclusa* produces seeds readily in the greenhouse, and seed production on native host plants is sparse, making extensive tests for seed transmission of the virus rather difficult for these two species.

PROPERTIES OF THE VIRUS

All property studies of the latent virus of dodder were made with juice from recently infected pokeweed plants. The juice, after being subjected to the various treatments, was inoculated into leaves of rapidly growing pokeweed plants by gently rubbing the inoculum over the surface of the leaves with a saturated cloth pad after the leaves were sprinkled with a small amount of abrasive. Results were determined from counts of the primary lesions. Three to 5 plants were inoculated in each test, and lesions on 3 consecutive leaves of each plant were counted.

Thermal Inactivation Point. Juice was placed in slender thin-walled glass tubes of about 1 cc. capacity. The tubes were immersed for a 10-min. period in water held at the desired temperature. Upon removal they were immediately immersed in cold water. They were then opened and the content used to inoculate plants. The results of 3 tests shown in table 2 indicate that the temperature of inactivation lies between 56° and 60° C., much of the virus apparently being inactivated at 58° C.

Longevity in Vitro. Juice was placed in small flasks and kept at laboratory temperatures (about 24° C.) until used. Inoculations were made at intervals shown in table 2. Results indicate that the virus was inactivated rapidly in expressed juice of diseased pokeweed. After 48 hours no infection was obtained.

Tolerance of Dilution. Juice from pokeweed was diluted with distilled water and inoculated into pokeweed leaves. Dilutions and results of 3 tests are shown in table 2. These results show that number of infections dropped off rapidly in dilutions above 1–50, but a few lesions were obtained from dilu-

TABLE 2.—*Properties of dodder latent mosaic virus as determined by local-lesion counts following inoculation to pokeweed*

Thermal inactivation				Longevity *in vitro*				Tolerance of dilution			
Temperature (10-minute period)	Average number of local lesions per leaf in trial No.			Period aged at 20°–24° C.	Average number of local lesions per leaf in trial No.			Dilution	Average number of local lesions per leaf in trial No.		
	1	2	3		1	2	3		1	2	3
°C.	*No.*	*No.*	*No.*	*Hours*	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*
Check[a]	19	30	48	Check[a]	42	23	28	Check[a]	28.4	36.0	46.3
54	7	23	44	2	27	26	34	1–10	28.8	27.7	40.0
56	3	16	21	6	10	25	58	1–50	20.6	18.1	20.3
58	0	1	0	12	4	6	31	1–100	8.2	2.3	9.2
60	0	0	0	24	2	1	2	1–500	.....	0.4	4.0
62	0	0	0	48	2	0	0	1–1000	1.2	0.4	3.3
				72	0	0	0	1–2000	.....	0.1	1.0
				94	0	0	0	1–3000	.....	0.1	0.3
								1–5000	0.0	0.0	0.0

[a] Fresh undiluted juice.

tions as high as 1–3000. Approximately the same results were obtained when juice from healthy pokeweed, instead of water, was used for making the dilutions.

Results of Other Tests of Properties. Other tests indicate that the virus retained activity in dried juice of pokeweed for less than 48 hours. No virus was recovered from dried leaves of pokeweed. The virus in juice of pokeweed passed readily through a one-half inch layer of Celite and through Berkefeld N and W filters with no detected decrease in concentration.

NAME AND DESCRIPTION OF THE VIRUS

The characteristics of the latent virus of dodder do not fully agree with those used in describing any of the 10 genera in the system of nomenclature and classification devised by Holmes.[1] On the basis of induced symptoms the virus agrees well with those placed by Holmes in the genus *Marmor,* but it does not conform to that part of the description of this genus that states regarding infected plants, "usually no recovery; if recovery occurs, no immunity to reinfection." However, in spite of the fact that host plants of the latent virus of dodder recover from symptoms and are immune from production of primary symptoms following a second inoculation, it seems best with this species to give major emphasis to induced symptoms and place it in the genus *Marmor,*[2] at least for the present. **Marmor secretum** sp. nov. (secretum, meaning hidden) is suggested as the Latin name of this virus. The following description of the species is presented.

[1] Holmes, F. O. Handbook of Pathogenic Viruses. Burgess Publishing Co. (Minneapolis, Minn.). 1939.

[2] It may be pointed out that the genus *Marmor* already includes at least 2 viruses (*Marmor rubi* and *M. persicae*) that cause primary symptoms from which there is a considerable degree of recovery. It seems doubtful whether in any case a plant has been shown to be immune from reinfection by any virus, although undoubtedly in some cases recovered plants are immune from production of primary symptoms following a second inoculation by the same or by a related virus.

Marmor secretum sp. nov. Attacks *Beta vulgaris* L., *Phytolacca americana* L., *Cucumis melo* L., *Fagopyrum esculentum* Moench, *Lycopersicon esculentum* L., *Solanum tuberosum* L., *Apium graveolens* L., and other plants. Typical symptoms consist of necrosis, chlorosis and mottling but all known susceptible species of plants partially or completely recover from symptoms of the disease. Produces primary lesions on inoculated leaves of pokeweed. Virus latent in *Cuscuta californica* Choisy, *C. subinclusa* Dur. and Hilg. and *C. campestris* Yuncker. Thermal inactivation point lies between 56° and 60° C. Inactivated *in vitro* at a temperature of about 24° C. in about 48 hours, withstands dilutions of 1–3000, retains activity in dried juice of pokeweed for less than 48 hours, and passes Berkefeld N and W filters.

No insect vector known. Transmissible to *Phytolacca americana, Beta vulgaris, Chenopodium murale* and *Cuscuta campestris* by juice inoculation. Also transmitted by *Cuscuta californica, C. campestris* and *C. subinclusa*. Transmitted through approximately 5 per cent of the seeds of *Cuscuta campestris* from diseased plants.

Descriptive Habitat. In *Cuscuta californica* on Box Springs grade 5 miles east of Riverside, California.

In the system of classification and nomenclature of Smith[3] the virus becomes *Cuscuta virus 1* and in the system of Fawcett[4] it should be designated *Cuscutavir secretum*.

DISCUSSION

At different times virus diseases have appeared in various parts of the world and swept rapidly over agricultural areas, sometimes causing enormous losses. Examples that may be cited are peach yellows in the northeastern part of the United States, curly top of sugar beets and other plants in western United States, and, more recently, mosaic of peach in the United States and yellow wilt[5] of sugar beet in Argentina. The source of virus for initial spread of these diseases to economic plants is more or less obscure in every case, but evidence indicates that the causal viruses were present in wild plants in the respective areas perhaps many years prior to their discovery on economic hosts.

The latent virus of dodder appears to present an example of a virus able to cause injury to several crop plants but has not yet encountered conditions that have permitted it to spread from wild to cultivated hosts and become established on the latter as a virus of economic importance. The virus probably has existed in native or introduced plants of Southern California for many years. But for the fact that it is transmitted readily to sugar beet by *Cuscuta californica,* probably it would have continued undiscovered so long as it remained restricted to its desert hosts.

Probably failure to find this disease on cultivated plants is due either to the absence of a vector able to carry it to cultivated plants or to the absence of a vector on such plants as are able to perpetuate the virus after it has made the transition from wild to cultivated hosts. So long as the present biological balance is maintained, it is reasonable to suppose that the virus will remain of no economic importance. However, the introduction of an insect able to function as an efficient vector of this virus might result in the sudden appearance and rapid spread of an entirely new virus disease on sugar beet, cantaloupe, celery, buckwheat, potato, and other crop plants.

[3] Smith, Kenneth M. A textbook of Plant Virus Diseases. P. Blakiston's Son and Co. (Philadelphia). 1937.

[4] Fawcett, H. S. Suggestions on plant virus nomenclature as exemplified by names of citrus viruses. Science, 92: 559–561. 1940.

[5] Yellow wilt is the name that in a forthcoming publication will be suggested for a virus disease that has caused serious damage to sugar beets from 1929 through 1941 in the Rio Negro valley of Argentina.

The presence of this virus concealed in desert plants, together with the evidence that a number of well-known viruses causing serious diseases of economic plants probably were derived from noncultivated species to which they caused little or no injury, suggests the possibility that innumerable other viruses may exist in wild plants of various parts of the world, many of which may have great potentialities for injury if the biological complex were so altered as to bring them into prominence on cultivated plants in their most destructive forms.

Although the disease caused by this virus is at present of no economic importance, the virus has certain characteristics that should be of interest to specialists. Among its interesting features is its failure to infect more than 4 of its 23 known host plants when introduced into plants by juice inoculation. Yet, one of its host plants (pokeweed) is so susceptible to infection by juice inoculation that numerous primary lesions are produced on inoculated leaves. As a rule, mosaic viruses that are transmissible by juice inoculation are transmissible by this technique to all or nearly all of their known host plants. However, with all such viruses the host range has been determined by juice inoculation supplemented in a number of instances by insect vectors and graftage. In the absence of dodder as an agent of transmission many of the plants now known to be susceptible to infection by the latent virus of dodder would be considered immune. The question arises, therefore, whether the latent virus of dodder is unusual in respect to the number of its host plants susceptible to infection by juice inoculation or whether the host range of many other mosaic viruses is imperfectly known because of the lack of, or failure to use, a method of inoculation that produces infection on all susceptible plants.

The fact that a mosaic virus exists that so far has produced infection only on a limited number of its host plants following juice inoculation, should offer encouragement for testing larger numbers of plant species for susceptibility to many viruses, such as those attacking members of the family Rosaceae, that are now considered nontransmissible by juice inoculation.

The latent virus of dodder is of interest also because of the initial severity of the disease that it produced on several of its host plants and the degree of recovery from symptoms that these plants attain in later stages of growth. Because of these characteristics and the accuracy with which relative virus concentrations may be estimated by the primary-lesion method using pokeweed, this virus may prove to be a valuable addition to the group of viruses now available for use in certain types of studies of recovery and immunological phenomena in plants.

SUMMARY

A new virus was discovered in dodder, *Cuscuta californica,* in the vicinity of Riverside, California, when dodder was removed from certain desert plants and established on sugar beet.

This virus produces mottling or necrosis, or both, on sugar beet, cantaloupe, tomato, potato, celery, pokeweed, *Chenopodium murale,* and other plants. All of the susceptible plants partly or completely recover from the more severe symptoms. Cantaloupe and buckwheat have shown the least recovery. *Cuscuta californica, S. subinclusa, C. campestris, Brassica incana* and certain species and varieties of *Nicotiana* are symptomless carriers of the virus.

No insect vector is known. However, the virus is transmissible by the 3 species of dodder, *Cuscuta californica, C. subinclusa,* and *C. campestris.* Transmission is readily obtained by juice inoculation to pokeweed on which the virus produces numerous primary lesions. It is transmissible less readily to *Chenopodium murale,* sugar beet, and *Cuscuta campestris.* No infection resulted from juice inoculation to any other species of plant. The virus was transmitted through slightly less than 5 per cent of the seeds of *C. campestris,* but not through seeds of cantaloupe, pokeweed, or buckwheat.

The thermal inactivation point of the virus lies between 56° and 60° C. Infection was obtained from juice of pokeweed diluted 1–3000. Activity was lost in juice of pokeweed in about 48 hours, in dried juice in less than 48 hours and no virus was obtained from dried parts of infected pokeweed plants. The virus passed Berkefeld N and W filters readily.

Dodder Latent Mosaic is suggested as the common name of the disease and the **Marmor secretum** sp. nov. is suggested as the Latin name of the virus.

Although this virus has not been observed to occur naturally on any crop plant, it is considered that with the introduction of an efficient vector the virus might be capable of producing a destructive disease on cantaloupe and buckwheat and a disease of lesser importance on sugar beet, potato, celery, and perhaps other plants.

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A SERIOUS STORAGE ROT OF CELERY CAUSED BY THE FUNGUS ANSATOSPORA MACROSPORA N. GEN.

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INTRODUCTION

In the muckland celery-growing areas of New York State a number of storage rots occur. Those caused by *Sclerotinia sclerotiorum, Botrytis cinerea, Erwinia carotovora,* and, occasionally, *Phoma apiicola* are already well known, but within the past decade another fungus probably has caused more loss to stored celery than any of the others. During this time it has been mistaken for *Phoma apiicola,* as described by Bennett (2), but recently its true identity as a fungus close to the genus *Cercospora* has been pointed out by the author (5).

RANGE AND ECONOMIC IMPORTANCE

Specimens and complaints have come chiefly from 6 cold storages in Wayne County, New York. Losses have been sustained, however, in a number of other counties, including Chautauqua, Niagara, Monroe, and Onondaga, and the trouble is known also in Ontario, Canada. Although these facts indicate a fairly widespread occurrence, nevertheless losses tend to be confined to the late-grown celery from certain particular farms or fields.

The disease seems not to have been described on celery, and there is a paucity of references to it in the literature on market diseases. Bratley and Wiant (1), in a summary report on the diseases of fruits and vegetables, found by them on the New York market during the last 3 months of 1939, state that, "Approximately 70 per cent of the stalks in 90 crates of upper New York State celery received in mid-December was found affected with a black crown rot. Although the symptoms were indistinguishable from those of Phoma root rot (*Phoma apiicola*) none of the isolates have developed fruiting bodies." C. W. Bennett, who described the Phoma root rot in this country in 1921, on examining a culture of this new fungus wrote the writer that it was definitely not *Phoma apiicola.*

VARIETIES ATTACKED

A number of standard types of celery have been grown 2 years on muckland farms, where the black crown rot was prevalent. None have been found immune. Late in October 1 or 2 crates of each variety were placed in commercial cold storage and held until January, when final examinations were made. In both years the celery looked healthy and free from rot at the time of harvest. In the first year it was tall and well-grown, but in the second it was stunted by the exceptionally dry season. In neither season could any evidence of crown rot be found before 7 weeks had elapsed in storage.

In the first test of 11 varieties from 15 to 50 per cent of the total of 1393 plants became affected in 10 weeks of storage. In the second test of 15 varieties from 9 to 65 per cent developed the rot in 11 weeks' time. There was no difference in susceptibility between green and yellow varieties. Easy Bleaching and Tall Golden Self Blanching were less affected than others in both tests, while Pascal and Golden Plume were among the

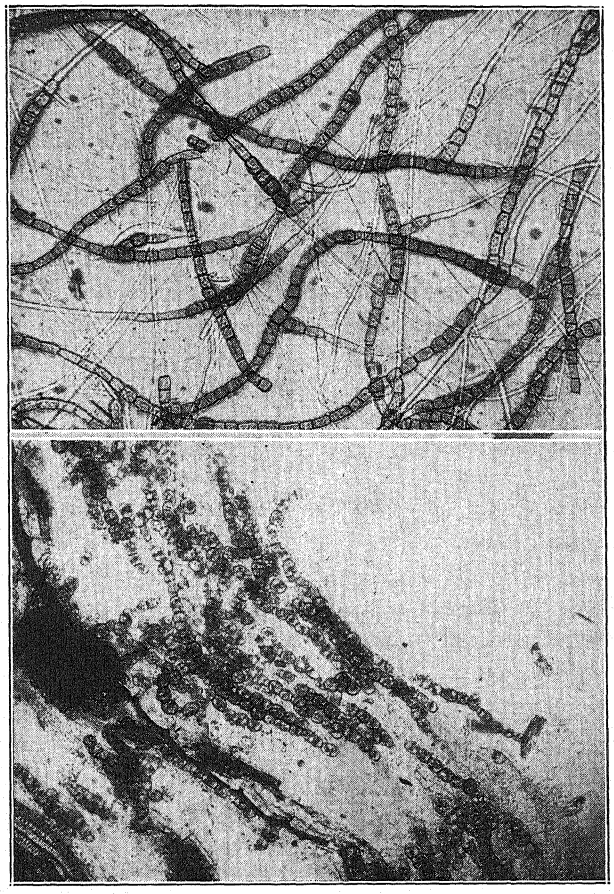


FIG. 1. Upper: An 8-day-old culture of *Ansatospora macrospora* on potato-dextrose agar, showing variable size of mycelium, the beginning of the torose condition and of deep coloration of older hyphae. ×85. Lower: Old mycelium of *Phoma apiicola* in celery tissue, showing its similarity to *Ansatospora*. ×85.

most severely attacked. The varieties tested were Easy Bleaching, Columbia, Utah, Tall Golden Self Blanching, Fordhook, White Queen, Old Golden, Golden Phenomenal, Giant Pascal, Meisches Special, Golden Plume, Abbott & Cobbs No. 14, White Plume, French Golden, Early Fortune, and Meisches Improved Golden. While the percentages of infection varied, the differences are not considered to be significant.

SYMPTOMATOLOGY

Diseased plants have rarely been found in the field. After celery is placed in cold storage, symptoms appear in 7 to 8 weeks, which usually means at sometime between November 25 and December 31. The first indication of infection is the appearance of a light ochraceous-tawny[1] colored lesion about 5 mm. deep somewhere on the butt end of the celery. As the brown rot advances further into the butt, the mycelium within becomes strongly torose and very dark colored (Fig. 1). This causes the color of the lesion to change from dull hazel to a dark olivaceous, which later becomes greenish slate-black[1] (Fig. 2, B and C). It is this ultimate greenish-black color that distinguishes the disease from other storage rots. The surface of the lesions often present a somewhat shiny appearance but it is not a particularly wet rot if unaccompanied by secondary bacterial pathogens. Later in the storage period, the characteristic dark lesions may be found at almost any point on the outer leaf stalks, indicating inoculation through wounds initiated possibly at harvest time. Conidia may be borne on the black lesions at room temperatures. Humidity is thought to be a very important factor in their production. Sometimes a distinctly red color may be imparted to the celery tissue near the outer margin of rapidly advancing lesions.

THE CAUSAL AGENT

Proof of Its Pathogenicity

Celery butts have been successfully inoculated with pure cultures of the black crown-rot fungus obtained by tissue plantings, and the fungus again has been recovered. Single-spore cultures obtained both from naturally infected celery plants and from agar cultures have proved pathogenic to celery stalks in cold storage and to celery seedlings grown under sterile conditions on agar, and to celery leaves. On the latter, lesions similar to early blight are produced. On their lower surface sporulation occurs if humidity is favorable (Fig. 3, A).

Cultural Characters and Morphology

The mycelium of the black crown-rot fungus is distinctive. It is not likely to be confused with any of the other common celery storage rotting fungi except perhaps *Phoma apiicola* (Fig. 1). At 18° C. on corn-meal agar a 3-inch Petri dish may be completely covered in 8 days, indicating a growth rate 3 or 4 times faster than that of either *Phoma apiicola* or *Cercospora apii*. Young hyphae are hyaline, mostly 3 μ in diameter, and are not conspicuously branching. After 3 to 5 days' growth on corn-meal or potato-dextrose agar at room temperatures older hyphae become 6 to 9 μ wide and soon develop some color varying between red, brown, and bluish green depending on the substrate. The cell walls thicken and the mycelium becomes strongly torose. The individual chlamydospore-like cells are at

[1] Ridgway, R. Color standards and color nomenclature, 45 pp. and 53 col. plates. (Washington, D. C.) 1912.

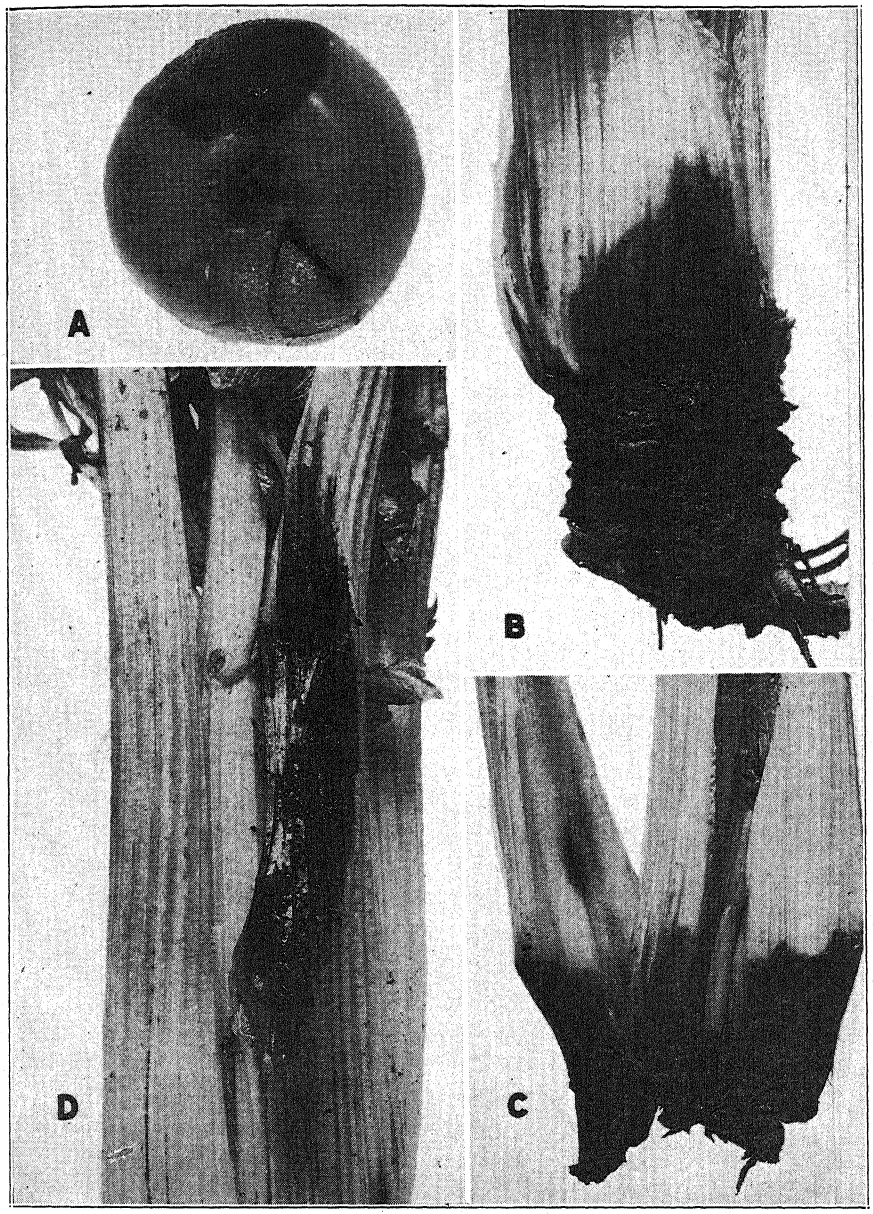


FIG. 2. A. An apple eighteen days after inoculation on the lower side with an actively growing culture of *Cercospora apii* and on the upper side with a culture of *Ansatospora macrospora* from celery. On carrots the same results were obtained. B. Typical crown rot symptoms, after 10 weeks in cold storage, involving entire crown and advancing up the leaf stalks. C. Late stage on celery as sometimes received on the market with butt end completely gone. D. Lesion on stalk near first joint where a bruise was probably sustained at harvest time.

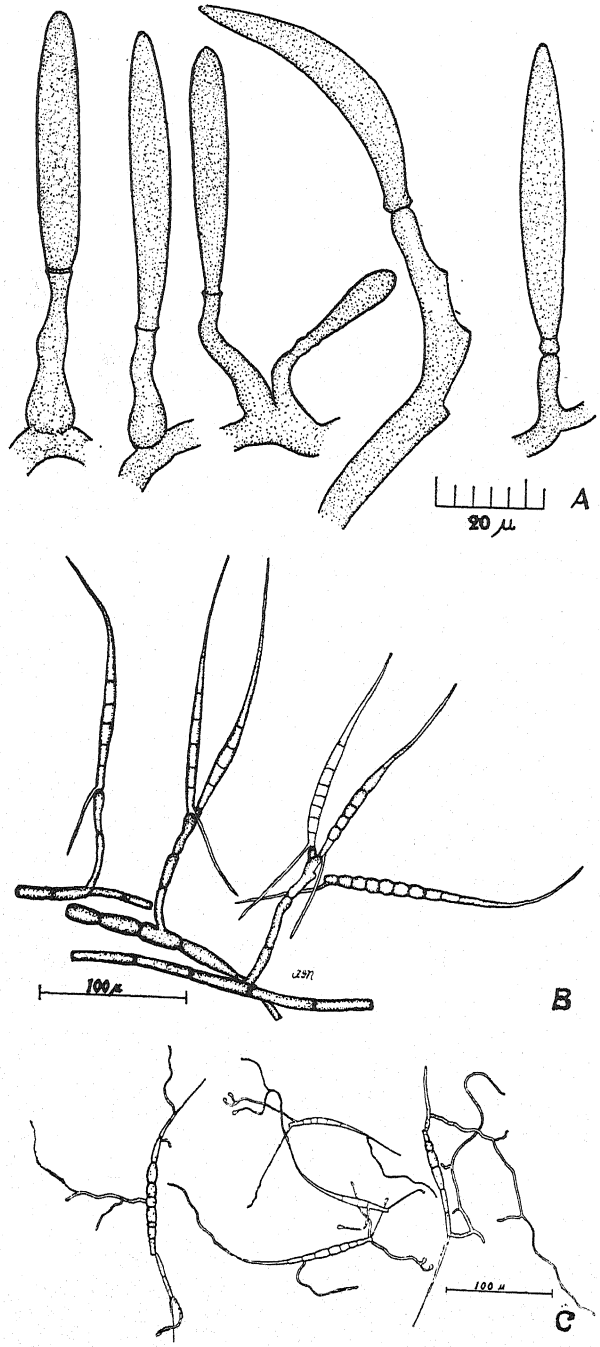


FIG. 3. A. Young conidiophores bearing conidia, from the lower side of a celery leaf spot 10 days after inoculation with a pure culture of *Ansatospora* from celery. B. Mature conidia borne on subhyaline conidiophores, from corn-meal agar culture. C. Conidia, germinating after 12 hours. A and C drawn with aid of camera lucida.

first 9 to 12 μ in diameter and later become 15 to 20 μ in 2 to 3 weeks with a few reaching 30 microns. They become ovate to spherical and very dark, olivaceous-brown to dark bluish or olivaceous-green. The same process occurs in the tissue of the host, which accounts for the distinctive color changes taking place there as the rot advances. On plain corn-meal agar, 6-day-old, rapidly growing cultures at 16° C. often develop a ring of bright red outside of the dark green central region. This has been seen also on diseased celery plants brought from cold storage to a more favorable temperature for rapid fungus growth.

Aerial mycelium is white and abundant, though not dense, on 2 per cent potato-dextrose or corn-meal agars. Alternate periods of desiccation and high humidity promote sporulation.[2] The subhyaline conidia are borne sparingly on potato-dextrose agar, more abundantly on corn-meal agar, after 30 days. More conidia and less mycelium are produced on potato and corn-meal agars low in sugars and much brighter red color occurs about the young hyphae.

The most abundant conidial production was obtained by employing the method mentioned by Westerdijk and van Luijk (6). After the fungus mat had nearly reached the sides of a Petri dish the agar was quickly inverted in the same dish. In a few days at room temperature sporulation occurred over the freshly exposed surface of the agar. Conidiophores arise singly or in clumps from young subhyaline mycelium. They are hyaline to subhyaline and bear 1 to several, falcate, hyaline to subhyaline conidia, 120 to 210 μ long (mean 160 μ), by 6 to 11 μ wide (mean 8.84 μ) (Fig. 3). Nearly half the spore consists of a long, tapering, whip-like beak, and a prominent, distinguishing feature on most spores, as they near maturity is a delicate, slender sword-like appendage 2 μ by 90 μ, which protrudes from the side of the basal cell at an obtuse angle. Westerdijk and van Luijk found some with 2 such appendages, but the author found more that seemed to lack them entirely. These conidia differ from *Cercospora apii* in being borne on shorter, nearly hyaline conidiophores, in being obclavate rather than acicular, in being twice the width, and in having the peculiar appendage just described. Germination occurs from any of the cells including the beak and tip of the appendage (Fig. 3, C). In culture they may germinate *in situ* and may anastomose freely.

Many efforts to induce the development of a sexual stage in pure culture and on celery stalks have been made but to no avail. The fungus, therefore, must remain at present as one of the fungi imperfecti falling in the Scolecosporae of the Dematiaceae in the key of Clements and Shear (3).

Identity of Crown Rot Fungus

In 1924, Osterwalder (4) described a leaf spot of pansy (*Viola tricolor*) caused by a fungus that seems to agree with the one described here. He called it *Cercospora macrospora,* although some of the conidia were not

[2] L. L. Hill, a graduate student, first found conidia during a routine classroom exercise in comparing the growth of several fungi isolated from celery.

strictly hyaline. In his illustrations the sword-like appendage is clearly visible, although he makes no mention of it. Specimens of a similar leaf spot of *Viola,* collected September 3, 1934, by G. F. Gravatt near Wrangell, Alaska, and sent to C. Chupp at Cornell, have been examined and found to agree with Osterwalder's description, except that conidiophores were not hyaline but brown. Conidia appeared identical with spores from the celery black crown rot fungus. Furthermore, in 1941, a culture that proved to be identical with the celery fungus was sent to Dr. Chupp by C. M. Tompkins of the University of California. This, it was stated, was obtained from a leaf spot of *Viola* on which the fungus was very destructive.

A few months after Osterwalder's description of his Viola disease in Germany, Westerdijk and van Luijk (6) described a fungus attacking caraway (*Carum carvi*) in Holland. The description fits that of *Cercospora macrospora* and the fungus causing black crown rot of celery. They mention the presence of peculiar appendages on the conidia and admit difficulty in assigning the fungus to any known genus because of them and of the variable color of the spores, which, on aging, do not remain hyaline. In naming their fungus *Cercospora cari* n. sp., they suggest that it probably deserves generic rank.

Since the writer has found the black crown-rot fungus from celery capable of causing lesions on caraway and on leaves of *Viola,* and the fungus from California has proved pathogenic to celery, there seems little doubt but that the cause of black crown rot is the same as the fungus causing leaf spot of pansy and anthracnose of caraway. If it were to be retained in the genus *Cercospora,* the name given it by Osterwalder, *Cercospora macrospora* Osterw., would stand. But, in a proposed monograph of the genus *Cercospora* by C. Chupp, no place exists for any forms having conidia with appendages of any kind. At his suggestion, therefore, a new genus, *Ansatospora*[3] is proposed for those fungi resembling *Cercospora* and *Cercosporella,* but whose conidia possess one or more appendages, cilia, or secondary conidia. The name proposed for the black crown-rot fungus is *Ansatospora macrospora* (Osterwalder) Newhall. Synonyms would include *Cercospora macrospora* Osterw. and *C. cari* (Westerdijk and van Luijk). The description follows:

Mycelium variable; at first hyaline, 3 μ in diameter, becoming darker, wider, and torose with age. Color becomes very dark olivaceous, while diameter increases to 15 or 18 μ. Conidiophores single or in small clusters, hyaline at first, 1–3-celled, geniculately bearing, terminally or laterally, 1 to several conidia. Conidia obclavate, falcate, hyaline to subhyaline, 120–210 μ long by 6 to 10 μ wide. Widest point a little below middle, 4 to 11 septa, truncate base, terminal, whip-like, beak 30 to 110 μ long. A straight to slightly curved whip-like appendage (rarely 2 of them) from 30 to 105 μ long by 2 μ wide, protrudes from basal cell at approximately a 45° angle with the main axis. Habitat, *Viola tricolor, Carum carvi* and *Apium graveolans,* causing leaf blight of pansies, anthracnose of caraway, and a crown and stalk rot of celery in cold storage.

Host Range

No attempt to explore the host range has been made beyond that deemed necessary to establish the identity of the crown rot pathogen. Small lesions

[3] Ansata = Latin for handle, hence spores with a handle-like appendage.

were obtained on the petioles of potted caraway and parsley plants grown from seed in the greenhouse. Similar lesions were obtained by inoculating leaves of *Viola tricolor*. When apples and carrots were inoculated with *Ansatospora* in comparison with *Cercospora apii*, only the former was able to infect (Fig. 2, A). This pronounced ability to cause rotting of vegetables and fruit at low temperatures is considered additional reason for separating *Ansatospora* from the typically leaf spotting Cercosporae. The suspicion that many other hosts occur has been confirmed by recent unpublished work of Tompkins and Hansen in California.[4]

EFFECT OF ENVIRONMENTAL FACTORS ON GROWTH AND PATHOGENICITY OF ANSATOSPORA MACROSPORA

Ansatospora macrospora, like *Phoma apiicola* in pure culture, has an optimum temperature close to 17° C. On corn-meal agar growth takes place between 0° and 27° C. but not at 31°. This low optimum and ability to grow at low temperatures partly explain the destructive action of this fungus in cold-storage celery.

Good growth of *Ansatospora macrospora* has been obtained on potato-dextrose agar adjusted to a pH ranging from 3.35 to 7.32. The optimum seems to lie very near the neutral point.

The fungus was found to endure desiccation in celery leaf stalks dried at room temperature for a period of at least 7 weeks, when the last of this tissue was pulverized, moistened with sterile tap water, and used to inoculate the butt ends of healthy celery placed in cold storage at 2–3° C. At the end of 11 weeks one of the 21 inoculated plants showed typical crown-rot lesions in which the fungus was clearly observed under the microscope. Three of the 4 plants inoculated with an actively growing agar culture of the pathogen became infected, and the 7 checks remained free from infection. It is not known whether conidia were present as part of the inoculum or not. Old dry agar cultures have been revived after 18 months of slow desiccation at room temperatures.

Some difficulty was experienced in early attempts to prove pathogenicity of *Ansatospora* for celery butts until adequate means were devised to maintain humidity in the experimental cold-storage chamber. An indication of the importance of this factor of humidity is afforded by the following experiment.

Three crates of celery from a supposedly infested field were brought to Ithaca and stored in a cold chamber at 32° F., where the humidity was approximately 60 per cent. A similar lot of 3 crates, placed in commercial cold storage, where the relative humidity was over 90 per cent, was kept under observation for over 2 months. At Ithaca 2, 4, and 14 per cent, respectively, of crown rot developed, while in the commercial cold storage 28, 42, and 47 per cent developed in the same period of time. Unfortunately, at the lower humidity the celery loses its turgidity, shrivels, and becomes

[4] The writer acknowledges their helpful criticism in the preparation of this manuscript.

practically worthless. Manipulation of the humidity factor, therefore, does not offer a hopeful approach to the question of control of crown rot.

CONTROL OF CROWN ROT

1. Fungicidal Dips at Harvest

On the assumption that crown-rot infections occur primarily at harvest time, perhaps from soil inoculum, a number of attempts were made to protect the butt ends of celery with fungicides as soon as cutting and trimming operations were completed in the field. Several fields were selected known to grow a crop that later developed crown rot in storage. Approximately 80 plants taken at random were trimmed and dipped for a few seconds in

TABLE 1.—*Results of celery butt dipping experiments for control of crown rot, after 9 to 11 weeks in cold storage*

Fungicide (strength)	Number rotting per crate					
	Farm					Mean
	A	B	C	D	E	
1. Lignosan[a] (1 part in 400 of water)	0	.....	.....	0	0	0.0
2. Ethyl mercury phosphate (2½ per cent) (1 part in 400)	2	0	2	0	0	0.8
3. Wet check—water	5	0	2	1	4	2.4
4. Dry check—no treatment	5	1	2	3	5	3.3
5. Copper carbonate (18 per cent) (1 part in 50)	8	0	6	3	0	3.4
6. Palustrex B[a] (6 tsp. in 2 gal.) = 1 per cent	4	1	8	1	0	3.5
7. Waxlac No. 69 + 1 per cent Palustrex B	.....	2	7	2	6	4.2
8. Waxlac No. 69[a]	7	0	.....	1	.....	3.6
9. Waxlac No. 69 + sol. of Hexamethalene tetramine ¼ per cent	.....	0	11	.....	3	4.6
10. Cuprous oxide suspension (1 part in 50)	2	0	8	8	2	4.0
11. Formaldehyde (1 part in 200)	1	4	12	6	6	6.4
12. Copper sulphate (1 part in 30)	2	24	17	29	11	16.6
13. Copper sulphate + starch (9 oz. each in 2 gal.)	6	23	29	.....	.....	19.0

[a] *Lignosan,* a powder containing ethyl mercury chloride 4.3 per cent; *Palustrex B,* a water-soluble pine oil containing 15 per cent copper-resinate; *Waxlac,* an emulsion containing carnauba wax, used in protecting nursery stock in storage.

a metal pan 3 inches deep containing one of the fungicides. The celery was then packed in crates holding 70 to 80 plants and placed in cold storage at 0° ± 1° C. where it was examined at weekly intervals beginning 7 weeks later. There were 11 liquid fungicidal treatments, besides one or two dry ones not reported on. The experiment was repeated on 5 farms, with slight modifications and included a total of between 4,500 and 5,000 plants. The materials used and the number of rotted plants per crate found, 9 to 11 weeks later, are given in table 1.

There were striking differences in appearance of the celery treated with the different fungicides. Those plants dipped in the organic mercurials were dry and clean looking and practically free from butt rot. Those dipped in the copper sulphate solutions were badly russeted, shrunken, and the prey of numerous molds. Several crates of celery treated with the carnauba

wax emulsion (Waxlac) developed green surface mold, which markedly lowered the appearance of the celery. The crates dipped in water were no worse than the dry checks, but, under field conditions, the 1-minute dip in water served to keep the butts wet only a few minutes longer than the so-called dry checks.

On 2 farms the butts were cut very short; in fact, flush with the crown. On 2 others a 1- or 2-inch stub of a tap root was left on, but seemed not to influence the subsequent amount of rot. It was concluded that, except for organic mercurials, the fungicides as used in these experiments were not able to prevent butt rotting. In similar tests dry sulphur and solutions of boric acid were tried and also found ineffectual.

To obtain light on the poisonous properties of celery receiving the mercurial butt treatments just described, half a bushel of butts were cut off and fed to guinea pigs and laboratory rats over a period of 2 weeks. This work was done in the laboratories of the Departments of Animal Nutrition and of Veterinary Pathology and Bacteriology of the New York State Colleges of Agriculture and Veterinary, respectively. During the feeding period supplementary foods were given these animals. They gained in weight and showed no outward signs of ill effects from the celery. At the end of the period after the animals had eaten more than their own weight of treated butts, the rats were autopsied. No internal symptoms of toxicity of any kind were found.[5] It is readily admitted, being negative data, that this is insufficient evidence to establish the safety of the use of a mercurial salt on such a crop of celery. The writer *emphatically does not recommend it,* even for butt ends that normally would be discarded. The apparent control obtained with such fungicides lends encouragement to the belief that infection occurs at the time of harvest and to the hope that some day an effective but safe treatment may be found.

2. Rotation of Crops

At present it appears that a rotation in which celery occupies the land once in 3 years is not long enough to eliminate this disease entirely. Celery growers are reluctant to adopt a longer one, however. The fact that carrot roots are also susceptible raises a question about the wisdom of growing this crop in the rotation on suspected land. Growers have obtained the most relief by growing only their early crops of celery on their suspected land, reserving the fields known to be disease-free for the late crop, which is likely to be stored. Crops harvested by September 5, usually are consumed long before crown rot can develop.

If celery from infested or suspected land is put in cold storage, it behooves the owner to begin examinations at weekly intervals after the 7th week. When a few soft butts can be detected by poking the fingers up between the bottom slats of the crates, the crop should be marketed within the next few days. It is when this precaution has been neglected that the greatest losses have been sustained, losses that have reached 100 per cent.

[5] Thanks are due Miss Gladys Sperling for her kind cooperation in making these tests.

COMPARISON OF BLACK CROWN ROT WITH OTHER STORAGE DISEASES OF CELERY

Since the black crown rot now occurs in important celery-growing areas of New York and Ontario, Canada, and since it may appear in other localities, a review of the essential characters by which it may be distinguished from the other principal storage rots is in order.

Mention has been made of the fact that symptoms are rarely found at harvest time and of the marked color changes that characterize symptoms of this disease, beginning with a light tawny brown and ending with its dark greenish black, sometimes bordered by a distinct red at the margin of rapidly advancing lesions. None of the other common celery storage rots caused by *Botrytis, Sclerotinia, Phoma,* or *Erwinia* goes through such a color range. Phoma root-rot lesions, with which crown rot may be confused, may advance up the leaf stalks but are a dark brown rather than a greenish black color; older crown infections present a rough, dry, cracked and distinctively scurfy surface. Numerous dark pycnidia often may be found by the aid of a hand lens on young as well as old affected plants. Under the microscope, lesions due to *Phoma,* like those of *Ansatospora,* are found to be filled with a dark torose mycelium nearly as large as that of *Ansatospora* (Fig. 1). But in culture *Phoma apiicola* grows only about one fourth as rapidly as *Ansatospora* and undergoes much less marked color changes. In New York the Phoma rot is an infrequent storage disease. It has been found occurring on seedlings and young plants as a severe root rot in the spring or fall. It has not persisted in the same field year after year, as the black crown rot has.

A summary of the distinguishing characteristics of the 5 principal storage rots is presented in condensed form as in table 2.

DISCUSSION

The origin of *Ansatospora macrospora* in New York State is unknown. The fact that Westerdijk and van Luijk found that it could be carried by the seed of caraway is suggestive. This host has long been grown in herb gardens in this State, and has escaped in places where it may now be found growing wild on waste land. Search for diseased "native" caraway plants has not been fruitful so far. Neither has the disease been found on pansies or other violas, although these are commonly grown. Other hosts may be found and their discovery may yet throw light on the origin and prevalence of the black crown rot of celery.

Since *Cercospora apii* is known to be carried very commonly in celery seed, it would not be surprising to find *Ansatospora* disseminated in the same manner.

Attention is drawn to the similarity between the leaf-spot symptoms of *Ansatospora macrospora* and *Cercospora apii.* Both fungi cause a brown necrotic lesion on the under side of which the conidia are borne. The possibility of *Ansatospora* being disseminated by air-borne conidia cannot be denied.

TABLE 2.—*Some distinguishing characters of five storage rots of celery*

Pathogen	*Ansatospora*	*Botrytis*	*Sclerotinia*	*Phoma*	*Erwinia*
Name	Black crown rot	Grey mold soft rot	Watery pink rot	Phoma root rot	Bacterial (slimy) soft rot
Occurrence	Storage and transit	Storage and transit	Some late field, mostly storage and transit	Mostly field, spring and fall, some storage and transit	Field, storage and transit
Gross host symptoms	Firm to soft, olivaceous-black crown and stalk rot	Soft to watery, tan to buff, crown and stalk rot	Watery, pink to creamy, crown and stalk rot	Scurfy, cracked firm, dark brown root and crown rot	Very mushy, wet rot, light-tan color and some odor at times
Signs of pathogen	None, though few conidia may show under microscope	Dense, drab-grey mold on older lesions. Black thin sclerotia *embedded in substrate*	Cottony white to pinkish surface mycelium. Black, thick sclerotia *on the mycelial surface growth*	Minute pycnidia with black papillae seen on older lesions with hand lens	Occasional soft greyish-tan, buttery glistening blisters filled with bacteria
Some cultural characters of pathogen	Mycelium variable in size from 3 to 20 μ, and in color from hyaline through *red, brown, green* to *olivaceous-black* on corn-meal agar or steamed rice. Older *mycelium strongly torose.* Covers Petri dish *in 8 days* at optimum temp. of 17° C.	Mycelium mostly 10 to 15 μ, hyaline to faintly colored, *conidia usually abundant*, giving drab color to culture, followed by *sclerotia closely embedded* in substrate. Optimum temp. 23° C.	Mycelium like *Botrytis* hyaline, *no conidia, sclerotia* more meaty and *occur superficially in the mycelial mat.* Optimum temp. 18° C.	Mycelium scanty variable in size and *becoming torose* like *Ansatospora* but *not as broad or as bright colored and takes 25 days* to cover Petri dish at optimum temp. of 16° C. *Pycnidia form in 5 to 10 days* on corn-meal agar	Typical bacterial colonies on agar

There is no evidence that the disease is picked up after the celery is brought to the cold-storage plants. There is little if any spread from one lot of celery to another, piled next to it, in the same room.

SUMMARY

The term "black crown rot" is proposed for a serious storage disease of the butt ends of celery causing considerable loss to growers and produce men in Western New York and Canada.

The disease is readily distinguishable from the other common storage diseases caused by *Botrytis, Sclerotinia, Phoma,* and *Erwinia,* by its ultimate black color, by its lack of aerial mycelium, or sclerotia, or pycnidia, or slime, by the presence of very dark olivaceous torose mycelium within the tissue and later of conidia sparsely borne on the surface of the black lesions.

The fungus causing this disease is thought to be the one previously described by Osterwalder as the cause of a pansy leaf spot, named *Cercospora macrospora* by him. Its identity with the fungus causing anthracnose of caraway, described and named *Cercospora cari* by Westerdijk and van Luijk also is established. Its name is changed to **Ansatospora macrospora** (Osterw.) n. gen. chiefly on the basis of the long, prominent appendage protruding from the basal cell of the conidium. A full description of the fungus is given.

Temperature limits for growth in culture were close to 0° and 31° C. with the optimum near 17° C. Good growth on agar occurred at hydrogen ion concentrations between pH 3.35 and 7.32 with the optimum near neutrality.

The fungus is capable of attacking leaves and stems of celery, stems and fruits of caraway, stems of parsley, and of rotting apples and carrots.

Circumstantial evidence indicates that black crown rot may be soil-borne. A 3-year rotation was not long enough to eliminate it from some fields.

Control by dipping the butt ends of freshly harvested celery in a number of different fungicides met with little success. Such treatment is not advised.

Since the disease rarely can be found until celery has been in storage for at least 7 weeks, growers have been successful in reducing losses by using fields, thought to be infested, for growing only their early crop of celery, which is never stored. Celery from infested fields if placed in cold storage should be carefully watched after 8 or 9 weeks, and, if symptoms appear, should be promptly marketed to avoid loss.

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THE RELATION OF SEED QUALITY TO THE DEVELOPMENT OF SMUT IN OATS[1]

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Satisfactory epidemics of seedling-infecting smuts are obtainable only by inoculating seed of a susceptible variety with viable spores of the pathogen under initial and prevalent favorable environmental conditions.

The spores must make contact with the caryopsis. For oats there are 2 methods: (1) Hull removal and dusting of caryopsis with chlamydospores; (2) Replacing by vacuum pump the air beneath the hull with a water suspension of chlamydospores. Both methods successfully place the inoculum upon the caryopsis, and epidemics are readily obtainable by either method. Many (1, 2, 3, 4, 6) have studied environmental factors governing infection. It is generally recognized that relatively dry soil at approximately 20° C. is most favorable to smut infection of oats, although moderate fluctuation of either factor does not prevent infection. Lods and Coulson (5) found that seedlings from small seeds of one variety were more susceptible to smut than those from large seed. The writer has confirmed their findings; and now reports quantitative results of more detailed experiments on plants from various seed lots of oats.

MATERIALS AND METHODS

Five varieties of oats were included in the experiments. Several seed lots were obtained of each variety (Tables 1, 2, 3), differing in place of origin and year of production. In 1940, one collection of *Ustilago levis* (Kell. and Sw.) Magn. and one of *U. avenae* (Pers.) Jen. were used, while in 1941 only *U. levis* was tested. With the exceptions stated in the text, seed was inoculated by the partial-vacuum method, a chlamydospore suspension of 0.5 g. smut in 100 cc. sterile water being used to inoculate 25 g. seed. In the few cases when the dry-spore method of inoculation was used, 0.5 g. of chlamydospores was used for 25 g. seed. Each seed lot was replicated 5 times in field experiments, 5 g. of seed being planted per 8-ft. row. Smut percentage was determined by head count, with an average of 200 heads per 8-ft. row, although older seed lots gave as few as 60 heads per row. The experiments on smut were made in the field at University Farm, St. Paul, Minnesota, in 1940 and 1941, while experiments on the relative germination rates of seed lots were made in the greenhouse at University Farm during the same years.

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Variability in Amount of Smut in Oat Varieties

In 1940, seed of Anthony C.I. 2143, Gopher C.I. 2027, Rusota C.I. 2343, and Iogold C.I. 2329 was inoculated with one collection, each, of *Ustilago levis* and of *U. avenae,* while only Anthony and Gopher and one collection of *Ustilago levis* were used in 1941 (Table 1). Similar results were obtained in both years. Plants from different seed lots of the same variety differ greatly in their reaction to smut. The seed lot of Anthony grown at St. Paul in 1939 produced plants that were only slightly smutted, while plants of the same variety from seed grown at St. Paul in 1936 were most heavily

TABLE 1.—*Percentage smut produced on plants from different seed lots of oats by Ustilago levis and U. avenae*[a]

Origin of seed lot		Variety, year of test, species of smut, and percentage of smutted heads									
		Anthony			Gopher			Rusota		Iogold	
Where grown	When grown	1940		1941	1940		1941	1940		1940	
		avenae	levis	levis	avenae	levis	levis	avenae	levis	avenae	levis
St. Paul	1935	26	46	61	.....	.....	.....	.....	.....	10	21
	1936	40	72	89	20	50	51	20	14	26	20
	1938	14	38	53	2	11	33	13	4	8	10
	1939	2	14	18	1	2	5	3	1	6	6
	1940	.....	.....	49	.....	.....	.....	.....	.....	.....	.....
Waseca	1935	.....	.....	.....	2	15	13	7	7	.....	.....
	1938	28	54	.....	1	19	23	6	3	2	10
	1939	16	43	80	1	9	17	5	3	1	3
Crookston	1937	16	45	65	1	16	14	11	5	3	10
	1938	10	43	73	1	7	9	2	2	1	3
	1939	10	37	82	1	7	12	4	3	1	3
Duluth	1938	13	42	75	1	6	8	7	4	0	0
	1939	3	22	54	0	2	4	1	1	0	1

[a] The writer wishes to express his deep appreciation of the kindness of H. K. Wilson, Division of Agronomy, University Farm, St. Paul, Minn., for supplying the seed lots used in this test.

attacked. However, some seed lots that gave plants only slightly smutted in 1940 produced more heavily smutted plants in 1941. Plants from seed lots grown at Waseca, Crookston, and Duluth, Minnesota, in 1939 were attacked more severely in 1941 than in 1940. Also, plants from the 1940 seed lot from St. Paul were significantly more severely attacked than plants from the 1939 St. Paul seed lot.

The varieties used in the above test were obtained from the supplies at the various branch experiment stations of the University of Minnesota and were supplied initially from the Agricultural Experiment Station at St. Paul. Because of the possibility that the lots obtained from the station in different years differed somewhat genetically, seed of Anthony and Black Mesdag oats produced at St. Paul in 1939 were sent to several stations in

the United States and Canada for increase. These 1940 seed lots[2] were inoculated with *Ustilago levis* and planted in St. Paul in 1941. Significant differences in the amount of smut were found in plants from the different seed lots. The percentage of smutted heads in Anthony ranged from 49 from the Texas lot to 83 from the Kansas lot, while in Black Mesdag the maximum amount of smut was 60 per cent from the Washington seed lot and only 7 per cent from the Texas seed (Table 2). However, there was only enough Texas seed to plant 2 replicates, and the plant sample may have been too small. However, the 28 per cent smut in plants from the South Carolina seed lot is significantly lower than the 60 per cent in plants from the Washington lot. Thus from one original seed lot of an oat variety, seed lots can be produced in different localities that give rise to plants differing widely in their susceptibility to smut.

TABLE 2.—*Percentage smut produced by Ustilago levis at University Farm, St. Paul, Minn., in 1941 on plants from seed lots of oats grown in different localities in 1940*

Origin of seed	Variety and percentage of smutted heads	
	Anthony	Black Mesdag
Texas	49	7[a]
West Virginia	50	52
South Carolina	52	28
Ohio	66	54
Alberta	75	43
Washington	81	60
Montana	82	46
Kansas	83	38

[a] Average of 2 replicates only.

Additional evidence to indicate that the differences between plants from various seed lots were not due to genetic differences was obtained from the testing of seed grown in 1940 from St. Paul seed lots of earlier years. In 1940, 4 seed lots of Anthony oats that were produced at St. Paul in 1935, 1936, 1938, and 1939 respectively, were inoculated and sown in the field at St. Paul. There were decided differences in the amount of smut in plants from the different lots (Table 1). In 1940 seed was obtained from these 4 lots of Anthony and inoculated with *Ustilago levis* in 1941, but there were only relatively slight differences in the percentage of smut in the plantings, the amount of smut varying from 30 to 45 per cent (Table 3). Seed produced in 1940 from the 1939 seed lot gave rise to plants more heavily smutted than plants from the 1939 seed lot, while seed produced in 1940 from the 1936 seed lot gave plants less heavily smutted than plants from the 1936 seed lot. Thus the susceptibility of plants from the 4 seed lots of Anthony appeared to result from the action of environment on the crop that produced the seed and not from genetic differences between the seed lots.

[2] The writer wishes to express his deep appreciation for the collaboration of the following men who in 1940 assisted in increasing the seed for this experiment: J. G. Leach, Morgantown, West Virginia; C. C. Allison, Columbus, Ohio; I. M. Atkins, Denton, Texas; T. W. Graham, Florence, South Carolina; L. E. Tyner, Edmonton, Alberta; C. S. Holton, Pullman, Washington; A. M. Schlehuber, formerly of Bozeman, Montana, and E. D. Hansing, Manhattan, Kansas.

TABLE 3.—*The amount of smut produced in 1941 on plants from four seed lots of Anthony oats grown in 1940 at St. Paul, Minn., the plantings having different amounts of smut in 1940*

Seed lots grown in 1940 from St. Paul seed lots produced in the years given below	Per cent smutted heads
1935	30
1936	45
1938	44
1939	31

A further test on the variability of oats to smut was available from seed grown at St. Paul in 1940. Seed of Anthony, Gopher, and Black Mesdag was sown at intervals during the spring of 1940 and seed from the plants grown that year was available. In addition, seedlings of these varieties had been germinated in the greenhouse and then transplanted to the field at intervals during the spring of 1940; and seed from these transplants also was available. The seed lots so obtained were inoculated with *Ustilago levis* and planted in the field in 1941. Only small differences in the amount of smut in plantings of these seed lots were found (Table 4).

Great variation in susceptibility to smut exists within plants from different seed lots of a variety of oats. Naturally, it is important in testing oat varieties to races of the oat smuts to recognize the possibility that variation in susceptibility of different lots may affect the relative susceptibility of varieties in different years. The average amounts of smut produced on Anthony oats by 6 collections of *Ustilago levis* at St. Paul, in 1939, 1940, 1941, and 1942, are 52, 8, 24, and 29 per cent, respectively, and with 5 collections of *U. avenae* in the same years, 48, 12, 16, and 30 per cent, respectively. The seed used in 1939 was from the 1938 seed lot, that of 1940 from the 1939 seed lot and, similarly, in 1941 and 1942, the seed from the preceding harvest was used. It was shown (see Table 1) that plants from the 1939 Anthony seed lot were smutted less heavily than those from 1938 and 1940 lots. It

TABLE 4.—*Percentage smut produced by Ustilago levis at University Farm, St. Paul, in 1941 on plants from seed lots of oats grown at different times in 1940 at University Farm, St. Paul, Minn.*

Seed lot derived in 1940 from		Variety and percentage of smutted heads					
		Anthony		Gopher		Black Mesdag	
Seed planted on	Seedlings transplanted[a] on	Seeded	Transplanted	Seeded	Transplanted	Seeded	Transplanted
April 24	April 23	61	36	9	14	40	37
May 3	May 2	35	41	14	11	47	45
May 9	May 7	49	66	12	7	43	41
May 18	May 16	64	46	9	7	38	41
May 25	May 25	33	29	9	11	36	32
June 10	June 5	.....	.....	4	5	25	17

[a] Seedlings transplanted to field from greenhouse when 5 to 8 days old.

would appear, therefore, that the seed lot of Anthony used in the 1940 smut tests was of great importance in causing the low amount of smut obtained in that year. Environmental conditions were not unfavorable to smut development in 1940, since Iogold was attacked at least as heavily as in most other years, and, indeed, in 1940, was more susceptible than Anthony.

It is important also to know, if possible, the reasons for the variation in susceptibility of different seed lots to smut. Two possibilities exist to explain these variations: (1) When inoculated with chlamydospores by the partial-vacuum method, different amounts of inoculum may get between the hulls and the caryopsis in different seed lots; (2) That the seed lots produce plants differing in physiologic potentialities that would determine the extent of smut attack.

Physical and Physiological Factors Influencing Smut Infection in Anthony Oats

Successful smut infection requires that the inoculum be in contact with the caryopsis. The seed lots used in the tests already described differed in

TABLE 5.—*The effect of dehulling seeds on and the relation of seed weight and volume to susceptibility to smut of plants from seed lots of Anthony oats*

Year seed lot produced at St. Paul, Minn.	Percentage smutted heads		Weight in g. of 1000 seeds		Volume in cc. of 1000 seeds	
	Normal	Dehulled	Normal	Dehulled	Normal	Dehulled
1935	61	74	15.1	8.8	50	24
1936	89	90	15.8	8.6	48	22
1938	53	75	14.1	9.6	46	22
1939	19	61	24.3	20.4	57	34
1940	49	77	17.5	13.4	49	26

relative weights and plumpness, and, hence, possibly would differ in the ease whereby chlamydospores could be placed adjacent to the caryopsis. The partial-vacuum method for smut inoculation replaces air between the hulls and the caryopsis by a water suspension of chlamydospores. Hence, differences in the degree of adhesion of the hulls to the caryopsis would affect the deposition of inoculum on the caryopsis. By inoculating the naked caryopsis, a standard quantity of chlamydospores could be applied.

Five seed lots of Anthony oats grown at St. Paul, in 1935, 1936, 1938, 1939, and 1940, were dehulled and the naked caryopsis inoculated by dusting with 0.5 g. dry chlamydospores per 25 g. seed, and the inoculated seeds planted in the field in 1941. The dehulled seed consistently produced more smutted plants than that inoculated by the partial-vacuum method, the greatest increase being in plants from the 1939 seed lot, in which 19 per cent smut was produced with the partial-vacuum method, while 61 per cent developed from dehulled seed inoculated with dry chlamydospodes (Table 5). However, the 1939 seed lot was still significantly less severely attacked than the 1936 lot, which had 90 per cent smut. Thus plants from seed lots

of one variety showed significant differences in response to smut when the seed was inoculated by the partial-vacuum method, while relatively slight differences were found following inoculation of the caryopsis with dry chlamydospores. It is probable then that the degree of adhesion of the hulls to the caryopsis is important in determining the spore load carried by a seed lot inoculated by the partial-vacuum method.

Since the opportunity for infection was similar for all seed lots inoculated with dry spores, the relatively smaller amount of smut in plants from the 1939 seed lot must be due to the physiologic response of this seed. Data on the weight and volume of the 5 seed lots were obtained (Table 5). Seeds of the 1939 lot were heavier and larger than seed lots of other years. It

TABLE 6.—*Relative germination rate of 3 seed lots of Anthony oats in steamed and field soil in greenhouse*

Treatment of seed		Seed lot	Field soil					Steamed soil				
			Stand[a] 12 days after planting	Per cent total stand, days after planting				Stand[a] 12 days after planting	Per cent total stand, days after planting			
				4	5	6	7		4	5	6	7
No inoculation	Normal seed	1938	62	0	60	81	86	104	2	47	72	76
		1939	210	21	82	92	94	204	22	77	89	95
		1940	129	11	55	72	79	168	7	44	61	81
	Seeds without hull	1938	40	8	56	80	83	92	14	62	73	87
		1939	145	57	85	91	95	208	49	81	89	93
		1940	82	17	51	73	81	156	26	55	71	82
Smut-inoculated	Normal seed	1938	87	6	62	85	90	111	6	36	70	83
		1939	196	24	81	90	91	196	14	79	91	93
		1940	133	8	50	68	83	177	4	54	69	79
	Seeds without hull	1938	14	0	36	57	71	82	10	65	88	94
		1939	126	41	77	84	88	194	55	82	89	92
		1940	43	7	49	65	86	172	23	66	83	88

[a] Total of 5 replicates, of 50 seeds each.

appears that plants from heavier seed tend to be less severely smutted than plants from lighter seed, this agreeing with the observations of Lods and Coulson (5).

In a preliminary report (9), the writer stated that the 1939 seed lot of Anthony oats germinated more rapidly than the other seed lots tested and, because of this more rapid germination, less time was available during which the seedlings could be infected. Germination tests on 3 seed lots of Anthony oats were made, the experiments being made in the greenhouse in field soil and steamed soil (equal parts of field soil and sand). Comparisons were made between normal and dehulled seed, and between inoculated and non-inoculated seed. Normal seed was inoculated by the partial vacuum method, while the dehulled seed was dusted with chlamydospores. Five replicates of 50 seeds each for each soil type and seed treatment were used, the seeds being planted uniformly at ½ in. depth.

In both steamed and field soil, the 1938 lot gave the poorest stand, while the 1939 lot was best. Little difference in stand appeared in steamed soil between normal and dehulled seed, whether inoculated or not, but in field soil dehulling of the seed greatly reduced the stand, a further reduction in stand occurring with smut-inoculated dehulled seed (Table 6). Tapke (7) has reported similar results. The relative germination rate of the 3 seed lots was similar on both soils and with all seed treatments, the 1939 lot in every case germinating more rapidly than the 1938 and 1940 lots (Table 6). In fresh weight and height, the seedlings of the 1939 lot consistently were heavier and taller than the 1938 and 1940 lots (Table 7). In general the

TABLE 7.—*Relative height and weight of seedlings from 3 seed lots of Anthony oats in steamed and field soil in greenhouse*

Treatment of seed		Seed lot	Seedling height in mm. and weight in g.[a]					
			Field soil			Steamed soil		
			Height in mm., days after planting		Average seedling test wt.	Height in mm., days after planting		Average seedling test wt.
			6	11		6	11	
					g.			*g.*
No inoculation	Normal seed	1938	20	58	0.141	23	58	0.140
		1939	37	87	0.240	29	73	0.227
		1940	25	73	0.207	25	72	0.171
	Seeds without hull	1938	21	50	0.138	24	37	0.150
		1939	42	74	0.246	37	79	0.224
		1940	22	53	0.187	31	74	0.180
Smut-inoculated	Normal seed	1938	21	53	0.114	21	56	0.132
		1939	35	72	0.267	32	70	0.236
		1940	29	70	0.201	27	72	0.164
	Seeds without hull	1938	13	32	0.177	23	56	0.156
		1939	33	70	0.245	36	77	0.201
		1940	19	46	0.170	33	73	0.158

[a] Seedling height measured from ground level to tip of uppermost leaf.

average seedling weight in field soil was somewhat greater than in steamed soil, and this difference may be due to the elimination of weak seedlings that would be more susceptible to attack of seedling-blight organisms.

It is apparent that plants of the 1939 seed lots as indicated by rapidity of germination, percentage germination of the seed and weight and height of seedlings were more vigorous than plants from the other two seed lots tested. Also, more vigorous seedlings tend to be less liable to infection by smut than weaker seedlings of the same variety. If vigorous seedlings tend to resist infection by smut more than weaker seedlings, then the 1939 lot, after storage for a period of years, should give rise to plants that are more readily infected. Tests run in 1942, with the 1938, 1939, 1940, and 1941 seed lots inoculated by the partial-vacuum method gave the following percentages of smut, respectively, 43, 25, 38, and 18. The differences in the

amount of smut in plants from the 1938, 1939, and 1940 seed lots planted in 1942 are therefore considerably less than the differences that were found in 1941 or between plants of the 1938 and 1939 seed lots in 1940 (Table 1). The relatively greater susceptibility of plants from the 1939 lot in 1942 in comparison with those from the 1938 and 1940 lots confirms somewhat the hypothesis given above, namely, that as seeds of a susceptible variety become older and become less vigorous in their germination capacity, they will produce plants more susceptible to smut.

DISCUSSION

During the past 10 years, field tests have been made at St. Paul to determine the pathogenicity of the collections of the oat smuts on several varieties of oats. It has been shown that some of the collections varied in pathogenicity on one variety in successive years and the variation was governed by the variety on which the smut inoculum had been increased (8). Obviously, different conditions at and immediately following planting will influence the degree of infection; therefore, the amount of smut on a susceptible variety should vary from year to year, depending on the occurrence of conditions suitable for infection. But the amount of smut formed also will be governed by the characters of the seed lot used. It has been shown that the seed-lot characteristics are of great significance in affording variation to attack by smut and that these characteristics may be as important as the environment under which the plants are grown in successive years.

The determination of physiologic races in the oat smuts, especially under field conditions, is complicated because plants from seed lots of the same variety of oats may be attacked by smut to different degrees. Any attempt to replicate in successive years the conditions of any one year, can approach success only if recognition be given to (a) the genetic stability of the smut collection, (b) the genetic purity of the host variety, (c) the requirement, as far as possible, of similar conditions of the soil in regard to temperature and moisture, at planting and for a few days thereafter, and (d) the utilization in successive years of seed lots producing plants that are attacked by smut to the same degree. The first two requirements are fairly easily solved; for smut collections, when purified by increase on one variety, tend to remain fairly stable, and standard varieties of oats show little variation. It is, of course, not possible always to obtain soil conditions favorable for infection. Nor can the variation of seed lots be readily controlled.

It has been shown that plants from seed lots of one variety are attacked more uniformly by smut when inoculation of the seed is accomplished by removal of the hull and dusting of the naked caryopsis, than when the partial-vacuum method of smut inoculation is employed. However, dehulling of seed is a slow and expensive procedure, and where many varieties are to be tested with several collections of smut, the more rapid partial-vacuum method is preferred. It is practical, however, to use one seed lot for a period of years for, although the viability of the seed will tend to decrease

with age, good stands can still be obtained with 5-year-old seed. If sufficient seed from one crop year can be obtained and used in determining physiologic races of smut for a 5-year-period, then yearly differences in the amount of smut produced will be reduced. The writer is of the opinion that the use of one seed lot of each variety for a period of up to five years will assist materially in interpreting the data obtained, since the variation in the amount of smut on plants from different seed lots will be reduced.

SUMMARY

Plants from seed lots of Anthony, Gopher, Rusota, Iogold, and Black Mesdag oats were tested for resistance to *Ustilago levis* and *U. avenae*.

Great variation in the amount of smut was found in plants from seed lots within one variety, the variation being as great as may be found between certain varieties.

It was shown that variation in the amount of smut occurred with plants from seed lots of one variety grown in different years in the same locality, and between plants from seed lots of the same variety grown in one year in widely different localities.

The differences in the amount of smut apparently are not due to genetic differences in the plants but to modifications in the seed resulting from the particular environmental conditions under which the seed was produced.

When seed lots were dehulled and inoculated by dusting dry chlamydospores on the naked caryopsis, smaller, although still significant, differences in the amount of smut were found than when the partial vacuum method of inoculation was used.

One seed lot of Anthony oats, when inoculated by the partial-vacuum method, produced plants with only 19 per cent smut, while 61 per cent smut was produced when the caryopsis was dusted with chlamydospores. In plants of other seed lots, only slight differences in the amount of smut resulted from the use of the two methods of inoculation. It is concluded that the relative tightness of the hulls surrounding the caryopsis is responsible for the difference in the amount of smut on plants from different seed lots.

The seed lot of Anthony oats that produced fewest smutted plants germinated more rapidly and gave larger seedlings than other lots of Anthony. It is concluded that the more vigorous seedlings of Anthony oats are less frequently attacked by smut than weaker seedlings.

It is considered that recognition should be given to differences in seed lots as a partial explanation of the variation occurring in smut tests made over a series of years. It is suggested that more uniform results in the determination of physiologic races of smut would be obtained by using seed from one harvest for a succeeding period of years rather than obtaining new seed lots each year.

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RECOVERY FROM CURLY TOP IN THE TOMATO IN RELATION TO STRAINS OF THE VIRUS[1]

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All varieties of *Lycopersicon esculentum* Mill. and of *L. pimpinellifolium* (Jusl.) Mill. are, so far as the writers are aware, susceptible to curly top. Under field conditions in the semiarid western United States, plants of certain varieties of tomato commonly recover from severe stages of the curly-top disease. The new growth of recovered plants shows mild curly-top symptoms, but if recovery be persistent and growth continue, the plants tend to become more nearly normal, so that late in the season they sometimes show only very slight evidence of the disease. This type of recovery has been observed only rarely in cultivated varieties of tomato, but in a primitive race of *L. esculentum* collected in Mexico and referred to as "Guasave A,"[2] a large proportion of the plants showed this reaction. Such plants, or cuttings grown from them, continued to harbor the virus of curly top, even though the plants were nearly normal in aspect. It was shown further that these plants were usually highly resistant to injury from reinoculation by means of the beet leaf hopper, *Eutettix tenellus* (Baker), the vector of the curly-top virus. Such plants are, therefore, considered to have attained a condition of acquired immunity similar to that developed in tobacco and tomato and described by Wallace.[3,4,5]

Each year for a period of several years, at the University of California Citrus Experiment Station, the writers have inoculated plants of Guasave A and its derivatives with the virus of curly top, and a detailed study of the reactions of these plants has been made. The reactions of this tomato race to curly top were not consistent from year to year, the time of the beginning of recovery, the proportion of plants recovering, and the degree and persistance of recovery, being highly variable in different years. For example, of 15 Guasave-A seedlings inoculated June 2, 1939, 14 plants recovered, 6 of the plants making a good, rapid recovery and becoming nearly normal in appearance. On the other hand, there was no case of recovery among 12 plants of Guasave A inoculated June 21, 1940. Two families, one of 27 plants and the other of 38 plants, derived from the backcross (Break o' Day × Guasave A) × Guasave A, were inoculated on June 6, 1939, and June 21, 1940, respectively. In the 1939 test, 16 plants (59 per cent) recovered; of

[1] Paper No. 496, University of California Citrus Experiment Station, Riverside, California.

[2] Lesley, J. W., and J. M. Wallace. Acquired tolerance to curly top in the tomato. Phytopath. 28: 548–553. 1938.

[3] Wallace, J. M. Evidence of passive immunization of tobacco, *Nicotiana tabacum*, from the virus of curly top. Phytopath. 30: 673–679. 1940.

[4] Wallace, J. M. Evidence of passive immunization of plants from curly top. (Abstract) Phytopath. 30: 26. 1940.

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these plants, 4 recovered early and made nearly normal growth. In 1940, only 6 plants (16 per cent) derived from the same backcross recovered, and these did not recover to a degree equaling that of the plants of the 1939 test. A similar variability was shown in an F_3 family from Riverside × Guasave A. Fifteen plants were inoculated in each year, 1939 and 1940. In 1939, 7 plants recovered, 2 of which developed into vigorous, almost symptomless plants. In contrast, only 2 plants of the 1940 group showed evidence of recovery, and both of these died after making a feeble recovery. By the chi-square test with Yates's correction, the odds against the occurrence, in random sampling, of the differences observed in these 3 pairs of values are about 24 to 1.

The influence of climatic conditions on the initiation and persistence of recovery is probably considerable. For the most part, tomato plants, even of varieties that have a tendency to recover, experience a severe initial shock following infection with curly-top virus. High temperatures speed the development of the disease and increase its severity. Under conditions extremely favorable for disease development, it appears that plants are often injured so severely and rapidly that there is not sufficient time for the reaction leading to recovery to take place and become effective. In the course of these investigations, however, the climatic conditions at the time of inoculation of the plants and during the periods following inoculation, probably did not differ sufficiently from year to year to account for all the observed variability with respect to recovery of the plants.

The discovery that differences in virus strains play an important role in the variability of acquired immunity from curly top in the tomato,[6] suggested that the variations in recovery in Guasave A and its derivatives, from year to year, might result partly from variations in the composition of the virus used for inoculation in these tests. The variations in recovery cited above occurred in tests made prior to 1941, when no special attention was given to the strains of virus used for inoculation of the plants. The plants were inoculated by means of leaf hoppers from many different stock colonies, some of which carried single, known strains of the virus, while others carried one or more unidentified strains.

In 1941 and 1942, tests with Guasave-A plants and known strains of the curly-top virus were, therefore, undertaken. It is the purpose of this paper to report the results obtained.

EXPERIMENTAL RESULTS

In 1941, 5 different strains of curly-top virus were used singly for inoculation of Guasave-A tomato plants. Virus strains 3, 5, 9, 58,[7] and 75 were selected because of their known virulence on cultivated tomato varieties.

[6] See footnote 5.

[7] Beet leaf hoppers carrying the individual strains of curly-top virus were supplied by N. J. Giddings, Senior Plant Pathologist, Division of Sugar Plant Investigations, U. S. Department of Agriculture, Riverside, California. Strain numbers above 10 have been tentatively assigned by him to strains under study but not yet given permanent numbers.

Five groups of 35 plants each were used. Each group was inoculated with 1 of the 5 virus strains, each plant having 15 leaf hoppers confined to a portion of the plant by a sleeve-type celluloid cage. The insects were caged on the plants on June 11, 1941, and the cages and surviving insects were removed 1 week later. General observations were made on the different groups of plants during the developmental period of curly top, and records were made of the incidence and severity of disease. The reactions of the plants in the various groups and the influence of virus strains on recovery are brought out in table 1. At intervals later in the season, after the plants began to show signs of recovery, the severity of symptoms on the original diseased parts of each plant was estimated, and the initiation and persistence of recovery, the amount of growth after recovery, and the degree of curly-top symptoms on recovered growth were recorded.

In 1942, similar studies were made on 3 groups of 35 Guasave-A plants each, inoculated on June 18, with curly-top virus strains 3, 9, and 58, respectively, which had also been included in the 1941 tests. Another group of 31 plants was inoculated by leaf hoppers from several stock colonies, each of which carried a different strain of curly-top virus. This population, composed of leaf hoppers carrying virus strains 3, 9, and 58, as well as several other strains known to infect tomatoes, was designated "composite strain mixture." A mixture of strains 3, 9, and 58 was used for inoculation of 5 additional plants: 5 leaf hoppers carrying strain 3, 5 carrying strain 5, and 5 carrying strain 58 were caged on each plant.

In both 1941 and 1942, there were some striking differences in the reactions of the Guasave-A plants inoculated with the different virus strains, and the differences observed between different strains were, in general, fairly consistent in the two years. In plants inoculated with a given single strain, however, the amount of injury before recovery, the time of initiation of recovery, and the degree of recovery were similar in the two years. In other words, the reaction of plants infected with a given strain in 1942 was similar to that of plants infected with the same strain in 1941.

As shown in table 2, the surviving plants in these tests were classified according to the amount of recovered growth, whether large, moderate, or small, and according to curly-top symptoms on recovered growth, whether mild, moderate, or severe. In interpreting these data, it must be kept in mind that there was considerable overlapping of the different classes. For instance, on some plants it was difficult to determine whether the amount of recovered growth was moderate or large and whether the symptoms were mild or moderate. The data show clearly that the strains of virus decidedly influenced the amount of recovery. With respect to the amount of recovered growth, plants infected with virus strain 3 were superior to all other groups. The severity of symptoms shown by the recovered plants in the strain-3 group was also, as a rule, noticeably less than that shown by the recovered plants of the strain-9 and strain-58 groups. Plants infected with strains 5, 9, 58, and 75, were, for the most part, in either the small- or the

TABLE 1.—*Development of curly-top symptoms and subsequent recovery, at Riverside, California, of Guasave-A tomato plants inoculated June 11, 1941, with different strains of the virus; 35 plants inoculated with each virus strain*

Date of observation and strain of curly-top virus	Number of plants affected	Original growth				Recovered growth	
		Symptoms	Stunting	Dieback	Fruitfulness	Stage of recovery	Symptoms
July 2, 1941							
Strain 3	34	Moderate	Medium	None	None	.....[a]	.....[a]
" 9	35	Moderate to severe	Medium to much	"	"	.....	.....
" 58	35	Moderate	Medium	"	"	.....	.....
" 75	32	Mild (as a rule)	Slight	"	"	.....	.....
" 5	3	Extremely mild	None	"	"	.....	.....
July 8, 1941							
Strain 3	35	Severe	Much	"	"	.....	.....
" 9	35	Severe	Much	"	"	.....	.....
" 58	35	Severe	Much	"	"	.....	.....
" 75	34	Mild	Slight	"	"	.....	.....
" 5	11	Very mild	None	"	"	.....	.....
July 30, 1941							
Strain 3	35	Severe	Much	Little	Little	Early	.....
" 9	35	Very severe	Very much	Much	Little	Early	.....
" 58	35	Severe	Much	Much	Little	Very early	.....
" 75	35	Severe	Slight	Much	Little	Extremely early	.....
" 5	26	Mild	Very slight to medium	None	Medium	.....	.....
Sept. 10, 1941							
Strain 3	35	Severe	.....[b]	Medium	———[c]	Very advanced, very conspicuous	Mild
" 9	35	Very severe	Very much	Much	———	Advanced, conspicuous	Mild to moderate
" 58	35	Very severe	Much	Very much	———	Fairly advanced	Moderate
" 75	35	Very severe	Medium	Much	———	Early, variable	Moderate
" 5	28	Mild to moderate	Slight	Little	———	Very early, inconspicuous	Moderate to severe

[a] Blank spaces denote that no recovered growth, and hence no curly-top symptoms on recovered growth, were apparent; or that recovered growth was too slight for recording of symptoms.

[b] Stunting difficult to estimate because of large amount of recovered growth.

[c] Dashes indicate no data.

TABLE 2.—*Influence of virus strains on recovery of Guasave-A tomato plants from curly top*

Virus strain	Year	Plants inoculated	Plants infected[a]	Plants died from curly top		Distribution of surviving plants according to					
				Before recovery	After weak recovery	Amount of recovered growth			Curly-top symptoms on recovered growth		
						Large	Moderate	Small	Mild	Moderate	Severe
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
3	1941	35	35	0	0	22	11	2	13	22	0
	1942	35	35	0	0	20	7	1	20	7	1
9	1941	35	35	2	2	8	13	10	9	19	3
	1942	35	35	5	1	3	17	3	0	20	3
58	1941	35	35	2	5	3	13	12	7	19	2
	1942	35	35	1	3	0	2	21	—[b]	—	—
5	1941	35	27	0	0	7	6	14	0	16	11
75	1941	35	35	2	3	9	13	8	4	24	2
Mixture of 3, 9, and 58	1942	5	5	1	0	0	1	3	0	2	2
Composite mixture (many strains)	1942	31	31	3	2	1	3	21	—	—	—

[a] Of plants infected in the 1942 tests, a number died from Fusarium wilt and are therefore not included in the data in succeeding columns of this table.

[b] Dashes indicate no data. The slight amount of recovery and the severity of symptoms on recovered growth made it difficult to distinguish between new and old growth.

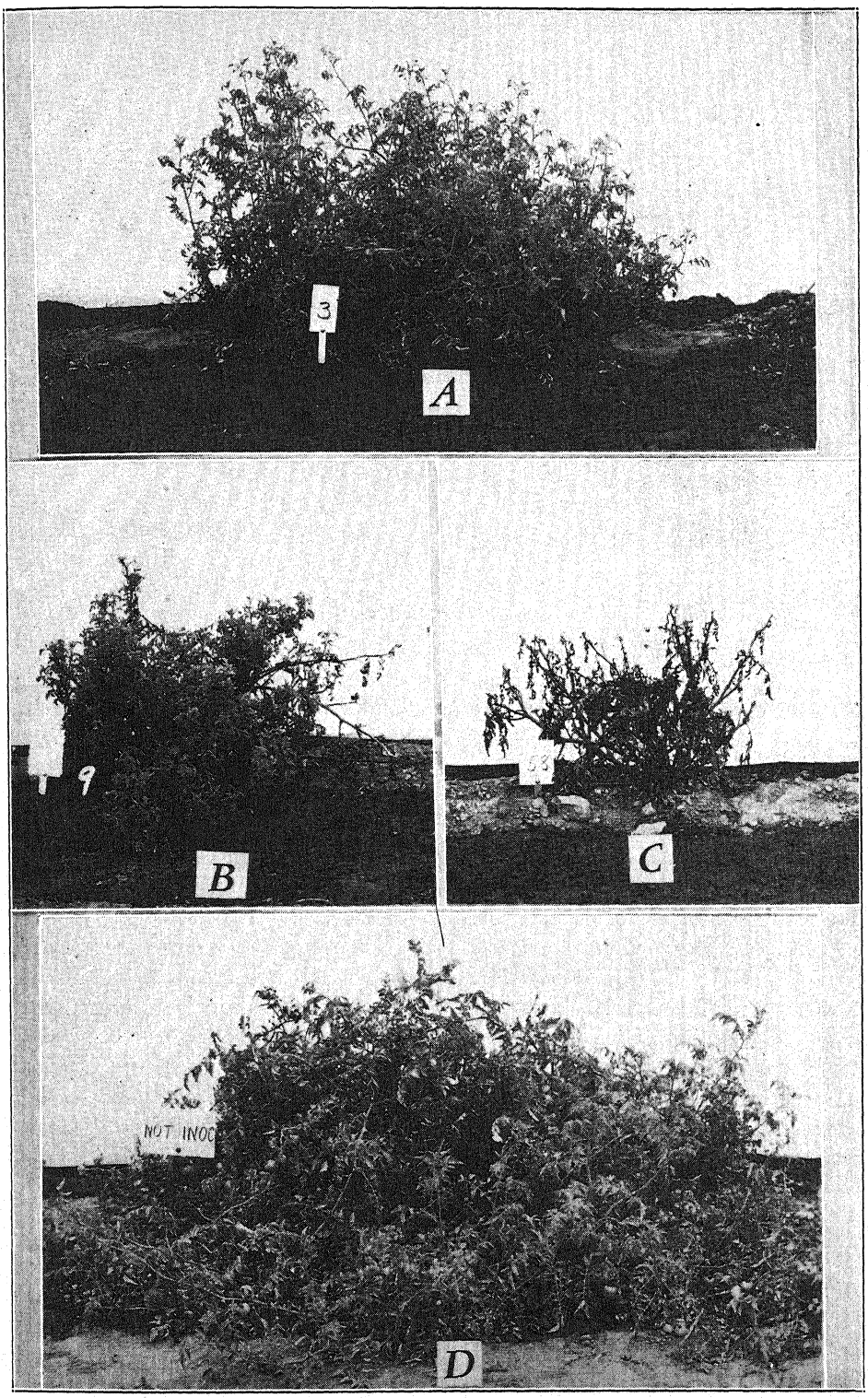


FIG. 1. The influence of different strains of the curly-top virus on recovery in Guasave-A tomato plants. A. Recovered growth of a plant infected with virus strain 3. B. Typical recovered plant infected with virus strain 9. C. Plant of group infected with virus strain 58, showing original severely diseased parts with slight recovery on the basal portions of the stems. D. Healthy, noninoculated plant. (Plants inoculated June 18, 1942; photographed September 17, 1942.)

moderate-growth classes; and comparatively few of the recovered plants of these groups were graded as having mild curly-top symptoms.

In the 1941 tests, recovery from strain 3 was more rapid, and the recovered growth was of a better quality, than in the case of plants infected with strain 9 or strain 58, even though all 3 strains caused severe injury during the early course of the disease. Recovery in plants infected with strain 9 was only slightly better than in plants infected with strain 58. The proportions of plants recovering were as follows: 100 per cent from strain 3, 88.6 per cent from strain 9, and 80 per cent from strain 58. Although virus strains 5 and 75 were not included in the 1942 tests, their behavior in comparison with that of the other strains, in 1941, gave further proof that, in this race of tomato, recovery varied with the strain of virus used.

In 1942, stunting of growth, due to curly top, was evident 18 days after inoculation. This was especially noticeable in plants inoculated with the composite mixture of virus strains and in those inoculated with the mixture of strains 3, 9, and 58. Plants inoculated with the individual strains were slightly less affected at that time. On August 31, 1942, 74 days after inoculation, recovery was by far the most advanced in the group of plants infected with virus strain 3, and was next best in the plants of the strain-9 group, which were, in general, in slightly better condition than those of the strain-58 group. On this date, the plants inoculated with the mixture of these 3 strains showed recovery about equaling that of the plants in the strain-58 group. Representative plants of the single-strain groups are shown in figure 1, A–C, in comparison with a healthy, noninoculated plant (Fig. 1, D). Earlier in the season, all the inoculated groups had shown very severe curly-top symptoms, consisting of almost complete defoliation and partial death of the stems. On September 16, 1942, the severity of the curly-top symptoms on the first-affected parts of the plants could still be estimated, except on the plants inoculated with virus strain 3, where those older parts were obscured by the large amount of new growth. In general, the condition of the recovered growth was more normal in plants that recovered earliest, and some plants, especially of the strain-3 group, produced a good crop of almost normal fruit on the recovered growth. Although all the different groups made more growth between the stage shown in figure 1 and the end of the season, the differences between the different groups remained in about the same order as that shown.

DISCUSSION AND CONCLUSIONS

In 1941, 5 strains, and in 1942 3 strains of the curly-top virus were used singly for inoculation of Guasave-A tomato plants. Since the percentage of inoculated plants that recovered was consistently high and probably not significantly different in the two years, it cannot be concluded that the variation in percentage recovery in 1939 and 1940 resulted solely from the use of different virus strains or strain mixtures. It is evident, however, that within a given year, Guasave-A tomato plants inoculated with different

strains of the curly-top virus varied widely in their recovery reactions. Even when the different virus strains caused severe initial symptoms resulting in marked injury to the inoculated plants, the time of the beginning of recovery and the amount and quality of recovered growth differed greatly. These differences were so great that it seems very probable that in some years environmental conditions may be the deciding factor in recovery of plants infected with some of the curly-top virus strains and yet may have very little effect on recovery from other strains. Recovery from virus strain 3 is initiated so rapidly and is of such high order as to suggest that, even under the conditions most favorable for development of curly top, plants affected by this strain would make a good recovery. On the other hand, in the case of certain other virus strains from which plants recover slowly and poorly, the same environment might cause a high mortality of plants, resulting in a low percentage of recovery.

In this study of recovery of Guasave-A tomato plants from curly top, the results support the earlier work of Wallace,[8] in which it was shown that acquired immunity from curly top in tomato may be of a high, intermediate, or low degree, dependent upon the virus strain carried by the immunized plants. Variations in recovery occurred between Guasave-A plants infected with strains of virus that cause equally severe symptoms on cultivated tomato varieties and similar initial injury on Guasave-A plants. It was of interest, however, that virus strain 5 was less virulent in its primary effect on Guasave-A plants than were the other virus strains used, but that recovery from strain 5 was inferior to that from strain 3, which caused severe injury in the early stages of infection.

While these investigations do not provide a conclusive explanation of the high interannual variability in the percentage of recovery of Guasave-A tomato plants, they suggest that the strain of virus, as well as the environmental conditions, determine the percentage of plants recovering. The data show further that, in investigations of recovery of tomato plants from curly top, it is essential that single virus strains, or known strain combinations, be used for inoculation of the plants under study.

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[8] See footnote 5.

A RAG-DOLL TECHNIQUE FOR THE INOCULATION OF WHEAT WITH BUNT (TILLETIA LEVIS)[1]

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(Accepted for publication April 21, 1943)

INTRODUCTION

The difficulty experienced in obtaining high percentages of infection of greenhouse germinated and inoculated winter wheat with bunt (*Tilletia levis* Kühn) has discouraged detailed studies involving the host and parasite. The common method of dusting the chlamydospores on the seed and then planting in moist soil frequently failed to produce infection high enough to permit use of the data. The same difficulty was experienced when seed was germinated on blotting paper and transplanted to soil. The possibilities of the rag-doll method (1) for the germination of wheat became apparent during studies of the development of amylase in germinating wheat (4). Accordingly, tests were started in the fall of 1940 to determine the feasibility of using a rag-doll technique for germination of bunt spores and subsequent inoculation of sprouting wheat.

Holton and Heald (2) have reviewed the conditions that influence the germination of bunt spores and the infection of wheat plants. They report numerous investigations in which the highest average infection was obtained when inoculated seed was germinated at 10° C. This temperature was favorable for the germination of the chlamydospores (2), and, according to Kneen, Miller, and Sandstedt (4), it is favorable for the germination of wheat in rag dolls. They found that wheat could be left in the dolls at 10° C. for as long as 10 days before the sprouts became too long for transplanting; at the same time the temperature was sufficiently low to prevent contaminating molds from becoming a problem. Consequently, the first inoculation test reported in this paper was conducted at 10° C. and later investigations proved this temperature to be the most satisfactory.

MATERIALS AND METHODS

The rag doll used was modified from that prepared by Duddleston (1) by omitting the paper from around the cloth. Wrapping the cloth in paper occasionally interfered with the germination of the bunt spores. When the doll consisted of just a roll of cloth, germination was consistently high and infection of the susceptible wheat seedlings was adequate.

The dolls, after sterilization for 30 minutes at 15 lb. pressure in the autoclave, were laid out on a clean table and thoroughly soaked with tap water; then the excess water was removed by lightly scraping the cloth with a flat, wooden pot label. A few dry, glass microscope slides were placed on the cloth and the dry bunt chlamydospores[2] were dusted on them and

[1] From the Departments of Plant Pathology and Agricultural Chemistry, Nebraska Agricultural Experiment Station; published with the approval of the Director as Paper No. 336, Journal Series.

[2] The chlamydospores were a composite collection of *Tilletia levis* made by K. S. Quisenberry from the bunt nursery at Lincoln, Neb., in 1940.

the rag portions of the dolls. The dolls were then rolled moderately tight and placed in constant-temperature cabinets at 10° C. The extent to which germination had occurred was determined daily by removing a slide from each doll and observing it under the microscope. The chlamydospores usually began germinating on the fourth day and the first primary sporidia were observed on the seventh day. Since Sartoris (5) concluded that only primary sporidia were capable of infecting the host, the wheat was not added to the doll until primary sporidia were being produced. In this manner the sprouts could be exposed to the sporidia for the maximum time at this temperature. The wheat was prepared for placing in the dolls containing the germinating chlamydospores by carefully removing all broken and badly shriveled kernels and then soaking the seed in distilled water for 18 hours at room temperature. No other seed treatment was used. Two hundred seeds were placed, 1 to 0.5 in. apart, on the portion of the cloth dusted with chlamydospores; the doll was then rolled fairly tight, returned to the constant-temperature cabinet, and left for 10 to 14 days, depending upon the rapidity of sprout growth. It was found that seedlings with sprouts 30 mm. long were readily transplantable, but, with longer sprouts, the labor and time required to prevent high seedling mortality increased rapidly.

Moisture and temperature apparently were the most important factors to control in the rag-doll method. In 2 separate tests the dolls were permitted to become fairly dry for a few hours, and in both cases low infection resulted. The best results were obtained when the dolls were kept near saturaton. Drying was retarded by placing pans of water in the constant-temperature cabinet. The moisture content of the dolls may be readily adjusted by sprinkling.

INFLUENCE OF TEMPERATURE ON THE GERMINATION OF BUNT SPORES

The influence of various temperatures on the germination of bunt chlamydospores in rag dolls was studied by dusting the cloth portion of the dolls with chlamydospores then placing the dolls in constant temperature cabinets at 5, 10, 15, and 20 degrees C. Germination was most rapid at 10° and 15°, slightly slower at 20°, and still slower at 5° C. The production of primary sporidia was favored by the 10° C. temperature, first being observed after 7 days. They were produced in the same time at 15° C., but in much lower numbers.

In another test at 10, 14, and 18 degrees C., germination was first observed after 5 days at all temperatures, being 12 per cent, 19 per cent, and 1 per cent, respectively. Primary sporidia were observed at all temperatures on the 10th day, and after 12 days there was a much higher percentage of spores with sporidia at 10° C. than at the other two temperatures. It was difficult to obtain accurate counts of spores with sporidia because the sporidia would often be separated from the mycelium when unrolling the doll.

INOCULATION STUDIES

The results obtained in 3 separate studies demonstrated that the rag-doll method of inoculation is practical in tests where seedlings can be trans-

planted after inoculation. Ceres spring wheat was used in the tests reported; similar results, however, have been obtained with Cheyenne and Blackhull winter wheats.

In the first two inoculation trials (Tests 1 and 2, Table 1) the seed was placed in the dolls on the 7th day following introduction of the bunt spores, when primary sporidia were being produced, and left in the dolls for 12 days before planting in soil. In test 3 (Table 1) the seed was placed in the doll on the 10th day and left in the doll at 10° C. for 12 days, when half of

TABLE 1.—*The degree of bunt infection obtained with Ceres spring wheat by using the rag-doll procedure for seedling inoculation*

Inoculation method	Number of plants headed[a]	Number of seedlings	Number of plants smutted		Percentage of plants smutted	
			Head count	Seedlings with chlorotic spots[b]	Head count	Seedlings with chlorotic spots
Test 1						
Rag doll[c]	127	127	115	109	95.5	85.8
Chlamydospores on seed[d]	130	130	42	47	32.3	36.0
Doll control[e]	88	89	0	2	0.0	2.2
Control—seed planted in soil	97	97	0	1	0.0	1.0
Test 2						
Rag doll	137	137	124	121	90.5	88.3
Doll—control	46	46	0	0	0.0	0.0
Test 3						
Rag doll—seedlings planted in sand	395	428	230	284	58.2	66.4
Rag doll—seedlings planted in soil	61	63	36	38	59.0	60.3
Control—seedlings planted in sand	36	36	0	0	0.0	0.0

[a] Due to some Helminthosporium seedling blight in test 3, a few plants died before heading. These were included in the seedling count.

[b] Chlorotic spots resulting from bunt infection as described by Johnston and Lefebvre (3).

[c] Bunt spores placed in rag doll and held at 10° C. until primary sporidia produced, then soaked Ceres spring wheat placed in doll until sprout was 30 mm. long. Sprouted seed planted in soil unless otherwise stated.

[d] Dry chlamydospores were dusted on the seed and the seed planted in flats of soil with a moisture equivalent of 12.76. The moisture was maintained at about one and one-fourth moisture equivalent and the flats kept in the greenhouse at 10° C.

[e] Seed germinated in doll, not inoculated.

the seedlings were planted in sand cultures and half in soil. Noninoculated controls, in which the seed was germinated in sterilized rag dolls, then planted in soil or sand, were used in each test.

The results of the inoculation tests presented in table 1 are recorded on the basis of the number of plants with smutted heads and the number of seedlings with the chlorotic spots typical of bunted plants. These chlorotic spots have been described by Tillet (6) and more recently by Johnston and Lefebvre (3).

There was a marked contrast between the amounts of infection obtained with the rag-doll inoculation and the procedure involving the dusting of chlamydospores on the seed and planting directly into the soil (Test 1). The low infection obtained by the latter method is typical of that obtained in previous years. The rag-doll method also gave a high percentage of infection in test 2, where the plants were grown in temperature cases at an air and soil temperature of 21° C. after the inoculation at 10° C.



FIG. 1. Stunting of wheat plants caused by bunt infection of seedlings and subsequent growth in sand culture. 7C—control. 7—inoculated.

The chlamydospores germinated slowly in test 3 and the infection was also reduced from the previous tests. The reason for the reduced infection is not known. However, the degree of infection was adequate for the experimental purpose. It made very little difference whether the inoculated seedlings were planted in sand or soil, except that the chlorotic leaf spots were evident earlier and were much more pronounced in the sand cultures. Under certain nutritional treatments in sand culture, stunting of the inoculated plants occurred. Figure 1 shows a comparison of the control and the inoculated plants grown under one of these treatments.

VALUE OF THE LEAF-MOTTLING CHARACTER

The chlorotic spots on the leaves of bunted seedlings were found to be valuable in making preliminary determinations of the amount of infection obtained. As can be seen from the table, there was not complete agreement between the number of seedlings with chlorotic spots and the appearance of smutted heads on the plants. A few of the seedlings showing the chlorotic spots that were typical of those on the leaves of bunted plants died from *Helminthosporium* infection, particularly in sand culture (Test 3). There were also a few cases in which it was difficult to distinguish between the spots and insect injuries. Then, too, it is entirely possible that the bunt infection may have encouraged the death of some of the seedlings before heads were produced. However, the data in table 1 indicate that with the variety Ceres it is possible to use the presence of chlorotic spots as an indication of the degree of infection and as a criterion on which to base continuance or early termination of an individual study. Similar data (unpublished) indicate the same to be true for two winter wheat varieties, Cheyenne and Blackhull.

SUMMARY

The rag-doll method of inoculation was found to be particularly well adapted to studies where wheat seed is to be germinated and inoculated with bunt under uniform conditions. A large number of seedlings can be inoculated with a minimum of labor and the resulting seedlings are in an excellent condition for transplanting. High degrees of infection were obtained by pregermination of the bunt spores in rag dolls followed by the addition of soaked wheat seed to the same dolls upon the appearance of primary sporidia. Seven to 10 days at 10° C. were required for adequate germination of the bunt spores followed by a 10- to 14-day period at the same temperature to permit the germinating wheat to produce sprouts of some 30 mm. length. It was found that such seedlings could be transplanted into either sand or soil with facility and in the case of the controls, with subsequent normal development.

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PHOMA TERRESTRIS ON GRAMINEAE IN THE NORTHERN GREAT PLAINS[1]

RODERICK SPRAGUE

(Accepted for publication August 4, 1943)

Kreutzer[2] demonstrated in 1941 that *Phoma terrestris* Hansen, which causes pink root of onion, could, under artificial conditions, attack certain cereals. In 1942, Fischer *et al.*[3] reported *Agropyron smithii* Rydb. as a natural host for *P. terrestris,* as determined by isolations made by the writer, and in 1943, Johann[4] listed corn as a host for this fungus. On the basis of studies during the past 4 years, it appears that *P. terrestris* should be recognized as a widespread but minor weak parasite and saprophyte on the underground parts of cereals, many grasses, and certain other hosts in North Dakota and adjoining States. During the 3-year period, 1940–1942, it was isolated 366 times, as well as a number of times in 1943.

During 1940 to 1942, the fungus represented only 2.3 per cent of 16,086 pure cultures of all species of fungi obtained by the writer from the roots of field-grown plants. However, while this fungus is seldom dominant in root material, it appears on a wide range of hosts. Isolations have been made from the following 55 gramineous hosts in this region: *Agropyron cristatum* (L.) Gaertn., *A. michnoi* Roshev., *A. repens* (L.) Beauv., *A. semicostatum* (Steud.) Nees, *A. sibiricum* (Willd.) Beauv., *A. smithii, A. trachycaulum* (Link) Malte, *Andropogon furcatus* Muhl., *A. hallii* Hack., *Arthraxon hispidus* var. *cryptatherus* (Hack.) Honda, *Avena sativa* L., *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis* (H.B.K.) Lag., *Bromus arvensis* L., *B. carinatus* Hook. and Arn., *B. erectus* Huds., *B. inermis* Leyss, *Dactylis glomerata* L., *Distichlis stricta* (Torr.) Rydb., *Echinochloa crusgalli* (L.) Beauv., *Elymus canadensis* L., *E. dahuricus* Turcz., *E. excelsus* Turcz., *E. interruptus* Buckl., *E. junceus* Fisch., *E. sibiricus* L., *Eragrostis pilosa* (L.) Beauv., *Festuca octoflora* Walt., *F. rubra* L., *Hordeum brevisubulatum* (Trin.) Link, *H. distichon* L., *H. vulgare* L., *Koeleria cristata* (L.) Pers., *Muhlenbergia richardsonis* (Trin.) Rydb., *Oryzopsis hymenoides* (Roem. and Schult.) Ricker, *Panicum capillare* L., *P. miliaceum* L., *P. virgatum* L., *Phleum pratense* L., *Poa pratensis* L., *P. secunda* Presl, *Roegneria pendulina*

[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Dry Land Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; and Division of Nurseries, Soil Conservation Service, U. S. Dept. of Agriculture; and the North Dakota Agricultural Experiment Station.

[2] Kreutzer, W. A. Host-parasite relationships in pink root of *Allium cepa.* II. The action of *Phoma terrestris* on *Allium cepa* and other hosts. Phytopath. 31: 907–915. 1941.

[3] Fischer, George W., R. Sprague, H. W. Johnson, and J. R. Hardison. Host and pathogen indices to the diseases observed on grasses in certain western States during 1941. U.S.D.A. Pl. Dis. Rptr. Suppl. 137. Aug. 15, 1942. (Mimeographed.)

[4] Johann, Helen. *Phoma terrestris* in the roots of mature maize plants. Phytopath. 33: 526–528. 1943.

Nevski,[5] *Schedonnardus paniculatus* (Nutt.) Trel., *Setaria italica* (L.) Beauv., *S. lutescens* (Weigel) F. T. Hubb., *S. viridis* (L.) Beauv., *Stipa comata* Trin. and Rupr., *S. viridula* Trin., *Sorghum vulgare* Pers., *S. vulgare* var. *sudanense* (Piper) Hitch., *Triticum aestivum* L., *T. durum* Desf., *T. dicoccum* Schrank, *T. timopheevi* Zhuk., and *Zea mays* L. We have also isolated *P. terrestris* from *Allium cernuum* Roth, *Cucurbita pepo* L., *Linum usitatissimum* L., and *Lepidium campestre* (L.) R. Br.

Although the roots of wheat growing in dry sandy soil in mid-late season afford the most common host for *Phoma terrestris,* it also is associated with a seed and seedling disease of corn, sorghum, and certain grasses earlier in the year. This latter condition was particularly common during June, 1943, at Mandan, N. Dak., when the precipitation for that month totalled 7.67 inches.

Trials in the greenhouse with soil-bran, pure-culture inoculum introduced at seeding time into electrically sterilized loam soil in redwood flats showed that *Phoma terrestris* is, at best, a very weak parasite. While strong pigmentation associated with mild lesions developed on the outer tissues of some hosts, no deep penetration of healthy tissue occurred. The first two triplicated inoculation trials were completely negative, except for superficial coloring of some of the roots and sub-crown internodes of *Agropyron cristatum, Elymus junceus, Poa pratensis* L., Rainbow oats, Wisc. 38 barley, and Pilot and Kubanka wheats. These were seeded in the greenhouse on Nov. 9, 1940, and examined on Jan. 20, 1941. The temperature in the greenhouse during this period ranged from 56° to 66° F. One of the two series was inoculated with *Phoma terrestris,* which had been originally isolated from Gopher oats, Dickinson, N. Dak., in July, 1941 (culture 212 A–1), and the other from Spartan barley (culture 214 A–1) from the same place. Both of these isolates had been obtained from maturing plants growing in dry soil. A third inoculation was made in the greenhouse in Nov., 1941, and grown at a slightly lower temperature (52° to 60° F.). The inoculated seedlings were examined Dec. 9, 1941. They showed the following reduction in yield of air-dried plants when compared with noninoculated healthy checks: *Agropyron cristatum,* trace; *A. trachycaulum,* 0.3 per cent; Rainbow oats, trace; *Bouteloua gracilis,* 10.5 per cent; *Bromus inermis,* trace; *Elymus junceus,* trace; Wisc. 38 barley, trace; Turghai proso, 1.3 per cent; Sudan grass, 4.4 per cent; Pilot wheat, none; and Golden Tom Thumb pop corn, trace. The culture in this third series was obtained from a seedling of *Agropyron smithii* at Mandan, N. Dak., May 15, 1941, and it was sufficiently pathogenic to merit listing in the 1942 check list[6] of the parasitic fungi on Gramineae. However, it should be emphasized that this fungus caused only 10.5 per cent loss in the small seeded grass, *Bouteloua gracilis.* Under the same artificial conditions, *Pythium arrhenomanes* Drechs. and *Pythium debaryanum* Hesse, usually caused complete preemergence death

[5] According to J. R. Swallen, *Roegneria pendulina* Nevski should be transferred to *Agropyron,* but the transfer has not yet been made.

[6] See footnote 3.

of this grass. *Phoma terrestris* is, therefore, considered to be a weak parasite on grasses and probably pathogenic only when the hosts are growing slowly in cool (about 50° F.) wet soil. The pink root condition in maturing cereals represents a saprophytic development on small roots killed by either *Pythium arrhenomanes,* other fungi, or by drought. It is evidently much less common than the pink root on mature cereals and grasses, induced by *Fusarium oxysporum* (Schlecht.) em. Snyder and Hans.

Phoma terrestris growing on potato-dextrose agar forms compact, slow growing, somewhat mounded velvety, gray colonies with rose-colored, plum or vinaceous tints, particularly in the substratum. The colonies are often sterile, particularly when incubated in total darkness in refrigerators. They vary considerably in amount of coloration and several distinct races are distinguishable. In 1940, J. E. Machacek told the writer that these cultures, which were sterile at that time, were probably a species of *Phoma.* In the fall of 1940, one culture isolated from *Elymus sibiricus* (199 A–2) produced elliptical, hyaline pycnospores, 3–4.8 × 1–1.5 μ. Later, typical cultures, obtained from *Agropyron cristatum, A. sibiricum,* and *Triticum aestivum,* and grown either on melilotus culms or on corn meal in flasks, produced spores similar to those of culture 199 A–2 when held under common storage conditions. The sterile, gray and rosy-tinted cultures are, however, so characteristic that little difficulty is encountered in distinguishing them from other fungi isolated from diseased roots of Gramineae.

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OBSERVATIONS ON A GALL OF SUGAR MAPLE

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(Accepted for publication June 1, 1943)

INTRODUCTION

Burls or galls of varying sizes and shapes are common on hardwoods. Certain of these burls are noninfectious and apparently result from injuries, while others are known to be caused by bacteria or fungi.[3] Globose or fusiform galls, often bearing one or more cankers, are fairly common on the trunks of sugar maple (*Acer saccharum* Marsh.) growing on the Kane Experimental Forest, Pennsylvania, and on the adjacent Allegheny National Forest. Similar galls also were observed in New York and New England. Lorenz and Christensen[4] have referred to the same type of sugar-maple gall as "globose canker." The cause of these galls is unknown, and the purpose of this paper is to describe them pending further investigations.

DESCRIPTION OF THE SUGAR MAPLE GALL

Sugar-maple galls were observed on trees from 2 to 18 inches d.b.h. with most affected stems from 4 to 8 inches in diameter. Practically all the galls developed on the main trunk, usually not over 6 to 8 feet from the ground. The number of galls on an affected tree ranged from 1 to as many as 4. If a sprout clump was affected it was not uncommon to find most of the stems in the clump bearing one or more burls. Trees with galls were not uniformly distributed throughout the forest, but usually occurred in groups of 2 or 3 affected trees in an area small enough to be readily noted from a fixed point of observation.

The gall starts as a localized outgrowth involving the whole diameter of the stem at the affected spot or only a part of the diameter, and results in a pronounced swelling (Fig. 1, A and D). In the early stages of gall formation the bark over the hypertrophied part is often smooth, or with only shallow ridges, and generally appears to be uninjured. On the larger galls the bark is usually cracked considerably on the part showing the greatest distortion. Dead streaks often develop on one or more sides of the swollen area, making the gall appear as a globose canker. The cankerous aspect of the galls usually increases with age, or at least with size, and the larger galls often have 2 or 3 open dead areas. Some of these dead areas finally heal

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[3] Brown, Nellie A. Tumors on elm and maple trees. Phytopath. 31: 541–548. 1941.

[4] Lorenz, R. C., and C. M. Christensen. A survey of forest tree diseases and their relation to stand improvement in the Lake and Central States. U. S. Dept. Agr. Mimeograph. 52 pp. 1937.

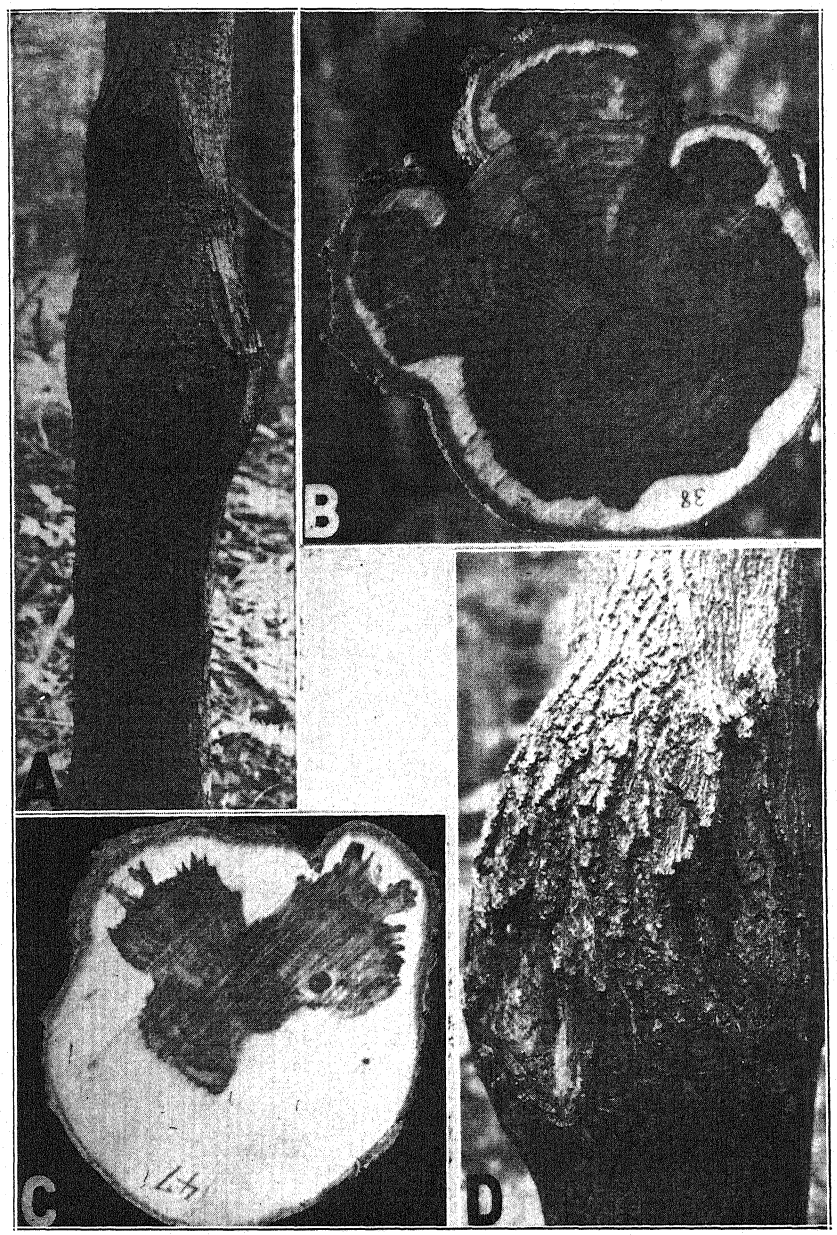


FIG. 1. Galls of sugar maple (*Acer saccharum* Marsh.). A. A typical swelling on young stem. B. Cross section of a gall showing extensive dark-colored internal area. C. Cross section of one-sided gall showing inner discoloration more extensive in swollen areas. D. A gall with two dead areas.

over, producing elongated ridges. The surface of such a gall seldom remains unbroken long, since further activity by the agent responsible for the swelling causes other dead streaks, cracks, or cankers to develop. In time, persistent dying of the cambium may kill the tree by girdling.

Cross sections through galls revealed that the wood associated with the hypertrophied part was dark, stained with greenish-black streaks or blackish streaks in a reddish-brown background in contrast to the normal white wood of maple. Sections through galls developing as one-sided swellings on the trunk illustrates this clearly, since the wood on the unaffected side remained white, while that at the center of the swelling was stained, the pattern of the stained part corresponding in general to the shape of the swelling (Fig. 1, C). Where the gall involved the whole diameter of the stem the discoloration extended throughout the entire cross-sectional area with the exception

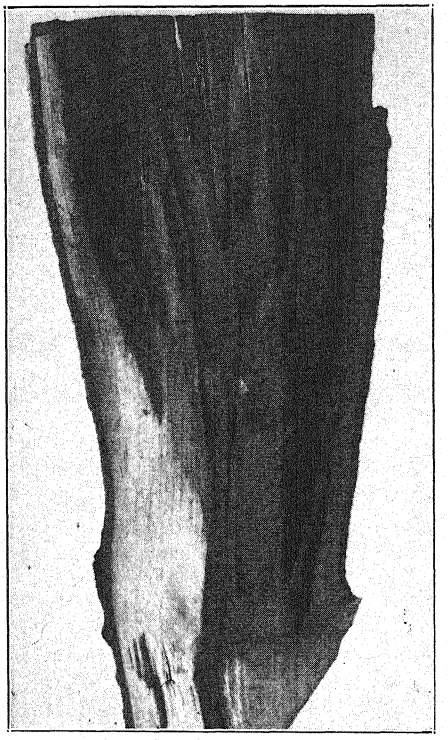


FIG. 2. Part of a longitudinal section of a small gall, showing restriction of zones of discolored wood to area of swelling.

of a narrow zone of sapwood (Fig. 1, B). On galls that had not developed extensive cankering it was observed that on the cross sections fingers of greenish stain extended toward the cambium from the mass of discolored wood at the center (Fig. 1, C). Wherever these fingers or outgrowths of stained wood touched or closely approached the cambium, the latter was killed, resulting in cracks or dead streaks on the surface of the gall (Fig. 1, B and D). The greenish or blackish discolorations extended only a short distance up and down the stem and were confined between the upper and lower limits of the swollen area (Fig. 2). This dark-stained wood remains very hard and heavy and is remarkably similar to what has been called mineral stain.

CAUSE

Numerous attempts to isolate a causal organism from the interior of this type of gall have given inconsistent results. No organism was obtained from the outer margin of the discoloration, that is, from the outer one-half to one-fourth inch of dark wood that was covered by a thin layer of living sapwood. Several organisms were usually obtained from the central area of discoloration. Most common among these were a slow-growing bacterium, similar to one isolated from redheart in birch,[5] and *Torula ligniperda* (Willk.) Sacc. Occasionally the conidial stage of *Coniochaeta* sp., a faster spreading bacterium, and *Coryne sarcoides* (Jacq. ex Fr.) Tul. were isolated from the same central or pith region of the discolored area. These organisms were like those consistently isolated from normal central discolorations in maples and birches.

The phomopsislike fungus reported by Brown[6] was never isolated from the galls. *Nectria* sp., although occasionally found fruiting on dead areas, was never isolated from the interior tissue.

There is usually very little evidence of a point of origin at the center of these galls, although it is supposed that there is some slight defect such as a small branch stub, insect injury, ring shake, or other dead areas as a point of origin.

If any of the organisms isolated is responsible for the death of the sapwood its effect must be indirect, because none of them was isolated from the advancing margin of diseased tissue. Another interesting point is that the pathological activity continues in narrow strips, on both sides of which dead areas have developed in the cambium (Fig. 1, B). The high moisture content of the stained wood might, through frost or extreme cold, have a detrimental effect on the living surrounding cells, but this has not been proved. Regardless of the actual cause, the gall appears to be caused by some infectious agent that gets into the stem when the trees are small and slowly works outward through the living sapwood and encroaches slowly to within one to several millimeters of the cambium with a resultant stimulation to growth at that point.

IMPORTANCE OF THE SUGAR MAPLE GALL

No estimate was made as to the percentage of affected trees on the Kane Experimental Forest, but the galls were "common," meaning that they occurred throughout the forest in numbers sufficiently great to be readily noticeable. Many of the trees with galls were in the intermediate or suppressed groups, and such trees usually are eliminated in the long run by natural thinnings. For practical purposes a tree bearing this type of gall has little promise of producing timber and may be removed if cuttings for chemical or fuel wood are being made. Evidently the agency inducing gall formation is not highly virulent, for the increase in their number in a stand takes place at a slow rate.

[5] Campbell, W. A., and R. W. Davidson. Redheart of paper birch. Jour. For. 39: 63–65. 1941.

[6] See footnote 3.

SYNERGISM AS A TOOL IN THE CONSERVATION OF FUNGICIDES[1]

ALBERT E. DIMOND AND JAMES G. HORSFALL

(Accepted for publication July 15, 1943)

Our country relies heavily on copper in the control of plant diseases. In the past year, 100,000,000 lb., computed as copper sulphate, were used in agriculture. Copper is much in demand in time of war. Plant pathologists are constantly on the alert to develop techniques for reducing the quantity used in combating plant diseases without sacrifice in level of control. It is the purpose of this note to point out the possibilities of using synergistic systems of fungicides to conserve copper.

For the past year the writers have been engaged in an examination of the extent to which savings in fungicides can be effected through synergism. A typical system showing synergism consists of carbon disulphide and dimethyl amine as members,[2] and results of a study of this system are presented in Table 1. The magnitude of savings possible through use of systems of poisons displaying synergism will be apparent.

TABLE 1.—*Relative dosage necessary to inhibit 50 per cent of a population of spores of Sclerotinia fructicola using a synergistic system*

Composition of poison		Relative quantity to give 50 per cent inhibition
Per cent Carbon disulphide	Per cent Dimethyl amine	
100.0	0.0	660.00
94.3	5.7	2.52
85.9	14.9	1.78
72.9	27.1	1.06
63.2	36.8	1.00
50.0	50.0	1.01
30.8	69.2	1.34
0.0	100.0	61.50

An illustration of synergism resulting from mixtures of two materials, only one of which shows toxicity to the test organism, is the system consisting of cuprous oxide and elemental sulphur. The dosages of these materials necessary to cause a 50 per cent inhibition of *Macrosporium sarcinaeforme* are shown in table 2. A distinction should be clearly made between synergistic and additive action of fungicides. That the system under discussion is synergistic rather than additive can be demonstrated from the fact that sulphur alone is not inhibitory to spores of *Macrosporium,* whereas combinations of copper and sulphur are more toxic than is copper alone. From the

[1] Contributions from the Department of Botany, University of Nebraska, N. S. No. 140, and of the Connecticut Agricultural Experiment Station. Published with the approval of the Director.

[2] The mechanism underlying synergism for this system is chemical, as will be shown in a subsequent paper.

point of view of the savings of total dosage of fungicide (copper plus sulphur), these data indicate that the phenomenon may be put to practical use, for, with this synergistic system, only half as much total fungicide is required for a given level of parasite control as when pure toxicants are employed. Furthermore, the savings in copper, the critical material in this system, is 10-fold.

Table 2 illustrates some interesting properties of synergistic systems. When both fungicides used in the system are plentiful, it will be most practical to utilize members at the composition that give maximum toxicity, *i.e.*, at the composition requiring a minimum total quantity of fungicide for a given level of parasite control. When one is plentiful and the other is not (and in this case cost is proportional to scarcity), it will be more economical and will conserve the materials needed in other ways to use the composition

TABLE 2.—*Savings in a copper fungicide made possible through using it as a member of a synergistic system against spores of Macrosporium sarcinaeforme*

Composition of poison		Total quantity of fungicide for 50 per cent spore inhibition (γ per cm.^{2})	Dosage of Cu_2O for 50 per cent inhibition (γ per cm.^{2})
Per cent cuprous oxide	Per cent sulphur		
100	0	5.00	5.00
97	3	3.41	3.31
90	10	2.50	2.25
70	30	2.12	1.48
50	50	2.12	1.06
30	70	2.27	0.68
10	90	4.93	0.49
3	97	75.80	2.27
0	100	Infinite	.....

giving a maximum saving of the critical material. The peak of toxicity as composition is varied will not necessarily be the composition most desirable from the standpoint of cost or of conservation of copper. In table 2, it may be seen that a composition consisting of 70 per cent copper and 30 per cent sulphur will be cheapest to apply and conserve the most copper.

It is evident that the savings of fungicide that can be effected through use of synergistic systems can be appreciable and may prove practical under field conditions.

The above data were obtained in the laboratory. Tests of synergism between cuprous oxide and sulphur as spray materials under field conditions in 1942 were made for control of potato tip-burn, a disease of uncertain etiology. Each mixture was applied in 3 different dosages, and each dosage was replicated in 5 randomized blocks (Table 3).

In Table 3 it is apparent that a mixture containing 75 to 90 per cent of cuprous oxide and 10 to 25 per cent sulphur gave the maximum protective value against tip-burn as compared with 70 per cent cuprous oxide and 30 per cent sulphur in the laboratory.

When considered in the light of the war-short material copper, the mix-

ture that used the least copper was that containing only 25 per cent cuprous oxide. Stated otherwise it means that if 75 per cent of the cuprous oxide was replaced with sulphur, the action of the cuprous oxide was synergistically improved some 14-fold. This is of the order of the 10-fold improvement in fungicidal action noted in the laboratory. Sulphur and copper have been used periodically together in sprays since Millardet first discovered Bordeaux mixture. In no case have the results distinguished additive from synergistic action. In the present case, sulphur alone was almost valueless in tip-burn control, so that synergism is clearly indicated.

No synergism between cuprous oxide and sulphur was observed in 1942 field tests on celery for control of *Septoria apii*.

Since there is at present no way of determining on an *a priori* basis what systems of poisons will be synergistic or the magnitude of synergism that

TABLE 3.—*Savings in a copper fungicide made possible through using it as a member of a synergistic system against potato tip-burn in the field*

Composition of poison		Dosage for 50 per cent tip-burn control (pounds per acre)	
Cuprous oxide (per cent)	Sulphur (per cent)	Mixture	Cuprous oxide only
0	100	224.00[a]	.....
10	90	19.40[a]	1.94
25	75	4.57	1.16
50	50	3.15	1.56
75	25	2.94	2.21
90	10	2.75	2.48
100	0	190.00	16.40

[a] Obtained by extrapolation.

will be shown by given poisons acting jointly, one can merely examine systems in a wholesale manner with the object of finding correlations and underlying mechanisms for the phenomenon. A considerable number of 2-member systems have been examined, and the following list indicates some possibilities: cuprous oxide with metallic oxides (ZnO, PbO, etc.), metallic oxides with sulphur, mercaptobenzothiazole with sulphur, and diphenyl amine with sulphur.

Yet another potentiality in the use of synergistic systems lies in the use of organic fungicides. An obstacle to the use of these materials as foliage sprays is their high cost and the fact that their production is limited by facilities of existing plants. Using organic fungicides as members of synergistic systems offers the possibility of reducing the cost of spray treatment and likewise of extending existing supplies.

Finally, it may be stated that systems of poisons synergistic for a single organism are not necessarily synergistic for all organisms. The system cuprous oxide-sulphur has been examined using two fungi: *Macrosporium sarcinaeforme*, susceptible only to copper, and *Sclerotinia fructicola*, susceptible to both toxicants. Preliminary results indicate that the increase in

toxicity against *Sclerotinia* is by no means so pronounced as it is against *Macrosporium*. Likewise, in the field, the cuprous oxide-sulphur system was synergistic in the control of potato tip-burn but not in the control of *Septoria apii* on celery.

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CRANBERRY FALSE BLOSSOM IN RELATION TO FLOODING WATER

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(Accepted for publication August 18, 1943)

The few Wisconsin cranberry marshes on which no case of the false-blossom disease has been observed on susceptible varieties during the past 7 years are chiefly those with alkaline flooding water. In at least 2 cases we have definite records of the introduction of infected vines of susceptible varieties on portions of such marshes where the disease is certainly less abundant than it was formerly.

This has occurred during a period when in spite of serious and apparently largely successful efforts at the control of the only known insect carrier, false blossom has in general increased on Wisconsin marshes. This opinion, namely, that, in spite of our best efforts at control, false blossom has been on the increase in Wisconsin during the past 7 years, is shared by

TABLE 1.—*Marshes with alkaline flooding water on which no false blossom has been found 1937–1943*

Location	Varieties	pH of flooding water
Hertel	Searls	7.6
Rice Lake	Bennett & Searls	7.4 – 8.8
Westfield	Searls	7.4 – 8.1
Berlin	Howes & Natives	7.5 – 8.2
Waupaca	Searls & Natives	7.8 – 8.5
Spooner	Searls	7.6
Spring Brook	Howes & Searls	7.5

other observers. It was, for example, voiced by Vernon Goldsworthy, who, as manager of the Wisconsin Cranberry Sales Company, has been a constant and keen observer of Wisconsin marshes for the last 10 years, in his Circular Number 40, dated June 23, 1941.

As is well known, false blossom is present on the vast majority of Wisconsin cranberry marshes. On the other hand, almost the only cases where marshes with very alkaline water are known to contain plants affected with false blossom are new marshes where false blossom plants have been recently set. The foregoing observations so strongly suggest a possible causal relation that they seem worthy of brief record, even though they are unsupported by experimental evidence. Moreover, if a causal relation could be demonstrated it would have no obvious commercial importance, for too many difficulties seem to be associated with the use of such water. The flooding water of the great majority of Wisconsin marshes is between pH 6.0 and pH 7.0.

Negative evidence, particularly negative field evidence, is usually not taken very seriously. The symptoms of cranberry false blossom are, how-

ever, conspicuous and the disease has been a major interest of mine for at least 20 years. It seems reasonably certain, then, that it is not abundant on these marshes, which have been repeatedly visited and studied during the period under discussion.

It is possible that the disease may never have been introduced on the marshes at Hertel, Westfield, and Waupaca. The only Searls vines planted on them are known to have come from what is still one of the cleanest marshes in the State. On the other hand, it is highly probable that diseased vines have been planted on each of the others and evidence that the disease was introduced on the Berlin Marsh in considerable amounts in vines of the susceptible Howes variety seems to be beyond question.

Under date of October 17, 1930, Mr. L. M. Rogers, then Cranberry Specialist for the Wisconsin State Department of Agriculture, made the entry in his notes with reference to this marsh, "False blossom well scattered through the Howes. I did not see it on the Natives." The Howes vines

TABLE 2.—*Marshes with alkaline flooding water where false blossom has been recently introduced*

Location	Variety	pH of flooding water
Hayward	Searls	7.9
Court O'Relles	Searls	7.2 – 7.9

here mentioned were planted about 1925, and came from New Jersey. They were almost certainly infected when planted. Later, under date of June 9, 1931, Mr. Rogers again notes the apparent absence of false blossom on the Natives and its presence in considerable abundance on the Howes. On August 30, 1933, May 17, 1935, and July 26, 1935, he mentions specifically looking for symptoms of the disease and failing to find them in the vicinity where they were found in 1930 and 1931. In the last record he adds "I think most of the false blossom that came in the Howes has died out." To this I can only add that I have been unable to find the disease here during 1937–1943.

There are other marshes than those listed that indicate alkaline flooding water does not favor the spread of cranberry false blossom. On the marsh near Turtle Lake (flooding water 7.4 – 8.0) there were planted in 1932, 5 acres of Howes vines known to have come from a marsh where there was and is a scattering infection of false blossom. Moreover, on August 31, 1935, L. M. Rogers entered the record "False blossom present in many places." Careful search on these same sections under favorable conditions in 1941, 42, and 43 revealed only a few uprights showing symptoms of false blossom. On a marsh near Nekoosa (flooding water 7.3), Mr. Rogers spent nearly an entire day in 1935 roguing false-blossom plants from a one-fourth acre section of somewhat mixed McFarlin vines planted in 1934. Since 1937, I have been able to find on this section only a single diseased upright. Even if it be assumed that Mr. Rogers removed every infected shoot but one in 1935, it is obvious that the disease has not spread.

The relation indicated by the foregoing observations suggests that these alkaline marshes, often not very productive of fruit, might be used for growing vines for sale as planting stock but for the psychological difficulties of selling vines from areas known to be relatively unproductive.

WISCONSIN STATE DEPARTMENT OF AGRICULTURE.

TILLETIA TUMEFACIENS, A REMARKABLE GALL-FORMING SMUT FROM INDIA

B. B. MUNDKUR

(Accepted for publication July 2, 1943)

Tilletia tumefaciens Sydow was founded by Sydow (1) to accommodate a smut occurring in the shoots and axillary buds of *Panicum antidotale* Retz. It was collected at Lyallpur on Sept. 30, 1908, and was sent by D. Milne to Sir Edwin J. Butler who diagnosed it as an undescribed species of *Tilletia*. The specimen in the Herb. Crypt. Ind. Orient. is a small portion of the

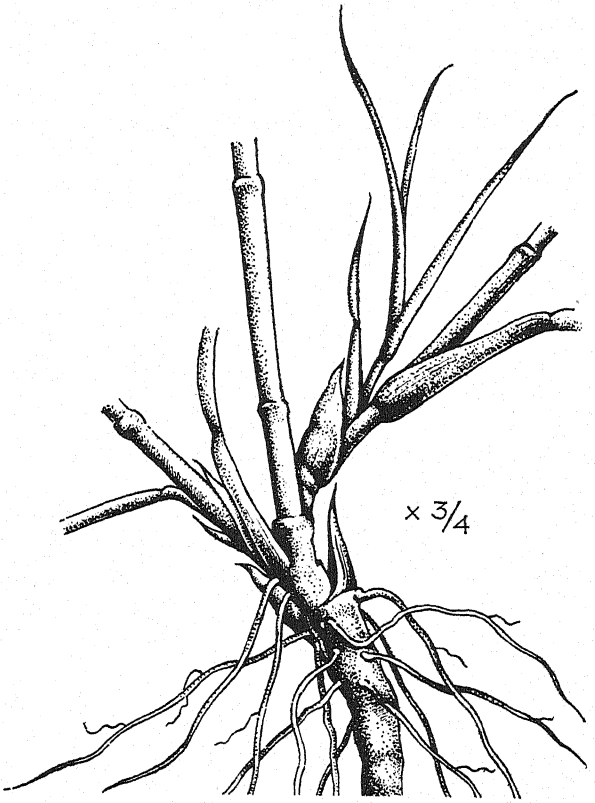


FIG. 1. Healthy *Panicum antidotale*, showing rhizome-like base.

scanty-type specimen, the major portion of which is in the Herb. Sydow at Stockholm. A description of the fungus recently was published by Mundkur (4).

Abundant material of this unique gall-forming *Tilletia* has recently been collected at Rohtak, a small township about 30 miles due west of New Delhi, which has enabled a more careful study of it to be made.

Panicum antidotale is a tall perennial grass with a stout rootstock; it is often found growing among the shrubs in hedges or as isolated bushes. Its

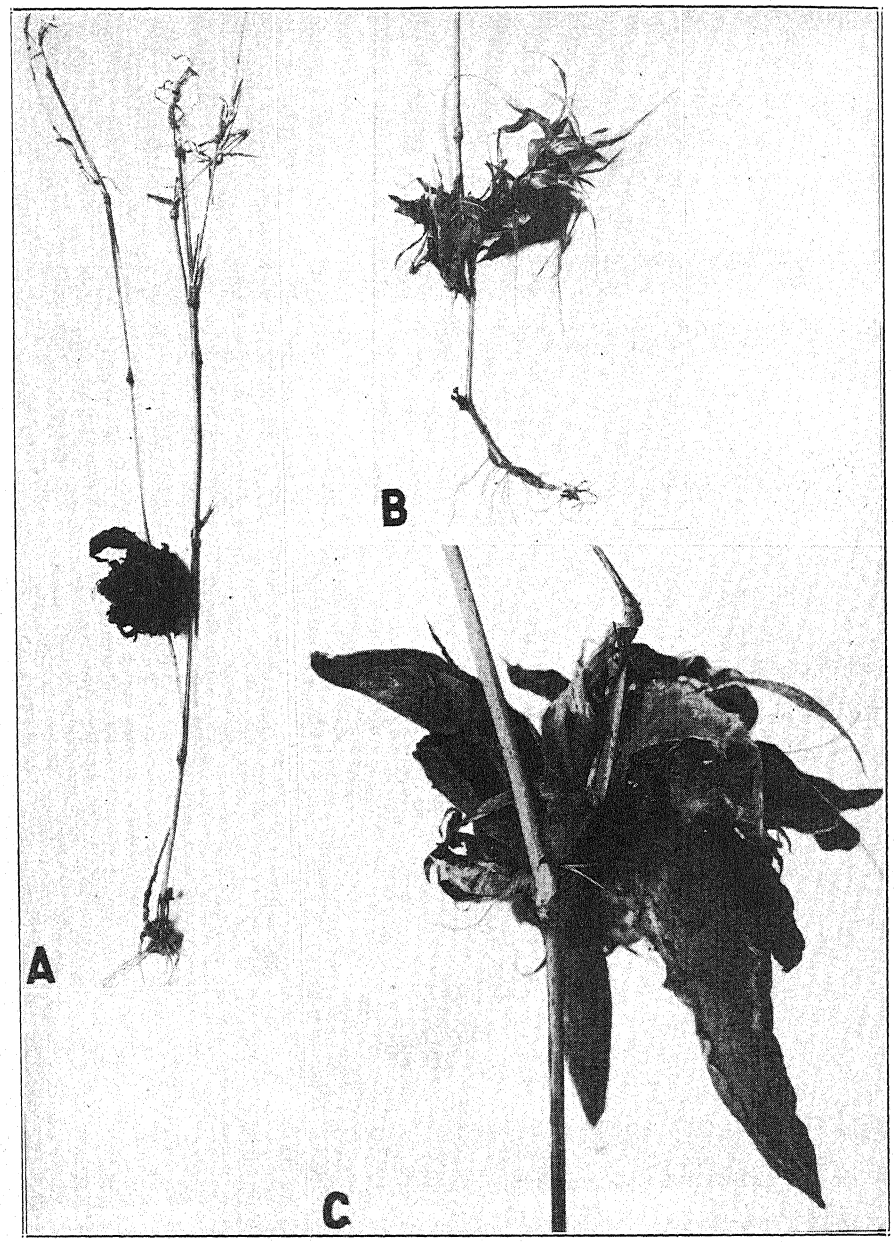


FIG. 2. A. Axillary buds turned into galls at the base of the plant. B. Witches broom effect; all leaves are not attacked. C. Buds transformed into large, finger-like tumors.

culms attain a height of 1½ m., are solid, terete and woody, and its leaves are rigid, linear, up to 60 cm. long and 2 cm. wide, with a smooth surface on both sides. Intra-vaginal branches arise from the leaf axils, especially at the base

of the plant (Fig. 1). The growing point of the young undeveloped branches is enclosed by smooth bud scales, about 2 cm. long. The grass is said to have a medicinal value in throat affections and for fumigating wounds; it also is stated to be an antidote for hydrophobia.

In a smutted plant (Fig. 2), the young axillary buds at the base are attacked, the main shoot, bearing undamaged inflorescence, being healthy. The upper axillary buds, as a rule, are not affected, though a gall also was found high up on the grass. The attacked bud, the bud scales and the prophyllum, as a result of hypertrophy and hyperplasia, become considerably swollen, forming a globose, black mass, having the appearance of a gall, though the structure actually consists of several galls held together. In advanced cases (Fig. 2, C), the central bud enlarges, being 9 to 10 cm. long and 2 to 3 cm. broad, forming a finger-like tumor. Within the tumor are the black spores, which easily get separated and dusted over the entire structure, giving it its black appearance. More than one bud in the axil may be attacked and 3, 4 or even 5 finger-like tumors can be seen emerging at one node. The scale leaves become hairy, swollen and considerably lengthened; they are filled within with the spores of the smut, the entire internal tissues being utilized by the fungus.

The masses of black spores are pulverulent and shed in enormous quantities. The galls emit the odor characteristic of wheat bunt, evidently due to the presence of trimethyl amine. The odor is so powerful that the presence of the smut can be made out even from a few feet away. The galls attract a considerable number of insects that carry the spores far and wide.

In the original description of the smut, it is stated that the sori develop in the apical region, converting the stem, bud leaves, and panicle into a large hood-shape gall. It will be noted that the abundant material that has now become available shows that the main stem is not attacked and that the parts where the sori develop are the axillary buds usually at the base of the plant. The spore masses, far from being rust colored, are almost black *en masse*, and the spores themselves are kaiser-brown to hazel (Ridgway). They are 16.0 to 23.0 μ in diameter with a mean of 19.3 μ. The mean of the measurements of 50 spores of the type specimen was reported by the writer (1940) to be 20.6 μ, but measurements of a larger number of spores now indicates that it is 19.3 μ. The sterile cells are thin-walled, smooth, 22.7 to 37.0 μ in diameter, hyaline, and occur in groups of 3 to 8. The epispore is 3 to 4 μ thick, with 5- to 6-angled reticulations. The frequencies are given below:

Diameter in μ	16	17	18	19	20	21	22	23
Frequency	9	20	32	75	28	22	9	5 = 200

Mean = 19.3 μ

Tilletia tumefaciens Sydow, loc. Rohtak, 27–12–1942, leg. S. Ahmad.

As far as can be ascertained from available literature, about 98 species of *Tilletia* have been described and, with very few exceptions, a majority of them are ovaricolous. In some cases the attacked ovaries are considerably swollen, but the formation of large tumors appears to be a character-

istic of *Tilletia tumefaciens* alone. Ciferri (1, 2) limits this genus to the ovaricolous smuts and has transferred *Tilletia earlei* Griff., which is culmicolous, to the genus *Ustilago*. However, *Tilletia flectens* Lagerheim, *Tilletia olida* (Riess) Winter, *Tilletia sterilis* Ule, *Tilletia Sesleriae* Juel are some of the well-known species whose sori are formed on the leaves in the form of long stripes and, even now (3), recognized as being well-established in the genus *Tilletia*. Limiting the genus to the ovaricolous species alone ignores the fact that the separation of the genera of the Tilletiaceae from the Ustilaginaceae is based, not on the location of the sori on the host, but on the mode of germination of the spores and other morphological characters.

The size and the general morphology of the spores of *Tilletia tumefaciens*, the place where the sori occur, the odor of trimethyl amine associated with it, and the mode of germination by formation of a nonseptate promycelium, at the apex of which the sporidia are borne, leaves no doubt that the limitation placed on the genus is not justified.

Specimens have been deposited in the Herb. Crypt. Ind. Orient.; Herb. Kew; Farlow Herbarium; and the Mycological Collections of the U. S. Dept. Agr., Washington, D. C.

SUMMARY

This note reports the rediscovery of the remarkable gall-forming species of *Tilletia* on *Panicum antidotale*, which Sydow named *Tilletia tumefaciens*. The limits of the genus *Tilletia* are discussed and evidence supplied to demonstrate that it cannot be confined to the ovaricolous smuts alone.

HERB. CRYPT. IND. ORIENT,
IMPERIAL AGR. RES. INST.,
NEW DELHI, INDIA.

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BOOK REVIEW

AINSWORTH, G. C., AND G. R. BISBY. *A Dictionary of the Fungi.* The Imperial Mycological Institute, Kew, Surrey, England. 359 pp. 138 figs. (ten plates). 1943. Price, $4.60.

It is now some years ago when I first learned of the intention of the Director of the Imperial Mycological Institute to publish a dictionary of the fungi—a sort of compendium volume to J. C. Willis' "A dictionary of the Flowering Plants and Ferns" (Cambridge, Univ. Press). All those familiar with Willis' very useful dictionary will agree that a similar one for the fungi would meet with a great reception, provided the task were accomplished in an equally creditable manner.

The book finally arrived and it affords me real pleasure to direct the attention of a wide circle of readers to same. Its aim has been nobly achieved, and, testing it from many angles, I have had no single disappointment. It is very much up-to-date. The generally unsatisfactory state of classification of fungi must have constituted quite a problem to the authors, who have, however, succeeded in not involving themselves—and the reader—in difficulties. It is surprising to note the vast amount of information the authors have been able to crowd into so little space. Many brief notes on subjects like: methods—bacteria—Ustilaginales—sex—pigments (chosen at random) have principal literature references added.

Last, but not least, the book is well got up—a credit to the printer and publisher, especially in these war times. It can be recommended without reservation as filling, most satisfactorily, a long-felt want. The authors are to be congratulated.— H. T. GÜSSOW, Associate Director, Science Service, Dominion Dept. of Agriculture, Ottawa.

NOTICE

A Statement of Recommended Procedure to be followed by an employer in requesting deferment of a Plant Pathologist has been prepared by the War Committee and distributed to "key" men in each State. If you do not have access to a copy of this statement, a copy may be obtained by writing C. C. Allison, Secretary, Dept. of Botany, Ohio State University, Columbus, Ohio.

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Yours sincerely

E. J. Butler

SIR EDWIN JOHN BUTLER, C.M.G., C.I.E., D.Sc., LL.D., F.R.S.
1874–1943

H. T. Güssow

Sir Edwin, notwithstanding a cordial relationship with numerous mycologists and plant pathologists on this Continent, never became a member of The American Phytopathological Society. However, many of us whose privilege it has been to know this eminent and lovable man, who after long and faithful service to our profession has passed from this life into one of everlasting peace and glory, feel that a place of honor may be fitly accorded him in our journal. Sir Edwin passed away on April 4th this year, after many years of loyal service in India, the climate of which undermined his health, as has so often been the case with many a stronger man. He had been very ill for the last two years—his heart grew steadily weaker—influenza set in, and the strain was too great. . . .

Born in County Clare, Ireland—educated at Queen's College, Cork—senior scholar in 1896—he was graduated in medicine (M.B. Hons.) at the Royal University of Ireland, but, like so many of his British colleagues of similar training, he favored Botany as his life's calling. During a travelling scholarship (1899–1900) in Paris, Freiburg—of which he often spoke with pride and pleasure—the Antibes, and Kew, he interested himself in the study of water moulds and related fungi.

In 1901 he received his appointment as cryptogamic botanist to the Government of India, and at once threw his heart and soul into the mycological problems of the great Empire he served with such distinction. In 1905 he went to Pusa as Imperial Mycologist at the Imperial Agricultural Research Institute, where his first task was to train workers in the methods of botanical and mycological research. He established for himself the reputation of a capable and inspiring teacher, commanding the respect and gaining the affection of a large staff who soon became devoted to this sincere, patient, and kindly man.

His earlier interests culminated in his still classical Memoir: "An account of the genus Pythium and some Chytridiales" (1907). With H. and P. Sydow he commenced a series of special articles (Fungi Indiae orientalis 1906–1916)—which laid the foundation to "The Fungi of India" published jointly with his good friend and collaborator, G. R. Bisby, formerly of the University of Manitoba but soon joining Butler's staff at the Imperial Mycological Institute at Kew. Among his numerous contributions to our science, his "Fungi and Disease in Plants" (Calcutta, 1918), perhaps more than any other, established his international reputation. Prior to his lamented death, Butler engaged in rewriting the first chapter for a new book he had long in mind, and in which he wished to summarize a life's long experiences.

The selection of so experienced and distinguished a man as first Director of the Imperial Mycological Bureau (now Institute) in 1921 was indeed a

wise and happy choice, and one that soon made the Institute, under his direction, one of the world's most famous. In 1922 this indefatigable worker, assisted by a devoted staff, brought out the first number of the "Review of Applied Mycology," which, as I have been told often by workers throughout the universe, "is the first periodical consulted when one takes up a new problem." The "Review" lives and surpasses in its scope and its conception similar publications in any language. In 1924 Butler inaugurated the first "Imperial Mycological Conference" between workers of the British Empire. During these, I became intimately befriended, enjoying often the great privilege of being his house guest and that of his charming lady. In 1926 Butler was elected a Fellow of the Royal Society of London (in 1934 to the council of the Society); in 1932 he was awarded the C.M.G.; in 1938 the old University of Aberdeen honored him with the degree of LL.D., and in 1939 he received his knighthood in recognition of his meritorious and outstanding services to his Empire. Finally, in 1935, he could no longer—as he told me—"in self respect" resist the urgent demands made upon him by the Agricultural Research Council of Great Britain and became its secretary. The destiny of the Institute, which he so successfully guided, then passed on to his trusted Assistant Director, S. F. Ashby, B.Sc., succeeded on the latter's retirement by Dr. S. P. Wiltshire, who has before him the task of carrying on in the spirit of its founder.

"Exegi monumentum . . . quod non . . . annorum et fuga temporum possit diruere."

MULTIPLICATION OF VIRUSES IN THE DODDER, CUSCUTA CAMPESTRIS

A. S. COSTA[1, 2]

(Accepted for publication September 16, 1943)

INTRODUCTION

Bennett (1) was the first to report that species of *Cuscuta* could be used to transmit certain plant viruses. Working with two species of this genus, *Cuscuta subinclusa* Dur. and Hilg. and *C. californica* Choisy, he was able to transmit cucumber-mosaic virus from tobacco to tobacco and also to *Nicotiana glauca* Grah. Moreover, he was able to transmit curly top of sugar beet by means of *C. subinclusa.*

The transmission of viruses through dodder was discovered independently by Johnson (3, 4), who succeeded in transmitting aster-yellows, tomato bushy-stunt, sugar beet curly-top, tobacco-mosaic, and white clover-mosaic viruses by means of the parasitic activities of *Cuscuta campestris* Yuncker.

Kunkel (5) reported transmission of cranberry false-blossom virus to several species of plants through the same dodder used by Johnson. He was the first to emphasize (6) that the great importance of this new method of transmitting plant viruses lies in the fact that many viruses heretofore obtained in a single or in few related species could by this means be transmitted to new hosts in which they could be studied to better advantage.

The role played by dodder in the transmission of viruses is not yet fully understood. Bennett (1) reported that *Cuscuta subinclusa* becomes infected with cucumber-mosaic virus and retains the virus indefinitely on immune plants. Johnson (4) pointed out that neither tobacco-mosaic virus, which was transmitted, nor tobacco-ring-spot virus, which was not transmitted, multiplies in dodder. No reference was made to the other viruses with which he worked.

The present paper reports results of a study of the role played by the dodder, *Cuscuta campestris,* in the transmission of 4 viruses: ordinary tobacco-mosaic virus (*Marmor tabaci* H. var. *vulgare* H.), aucuba-mosaic virus (*M. tabaci* H. var. *aucuba* H.), cucumber-mosaic virus (*M. cucumeris* H. var. *vulgare* H.), and cranberry false-blossom virus (*Chlorogenus vaccinii* H.).

MATERIAL AND TECHNIQUE

The experiments herein reported were conducted in one of the greenhouses of The Rockefeller Institute for Medical Research, Princeton, New Jersey, from January to June, 1943.

[1] Fellow of The Rockefeller Foundation.

[2] The writer is greatly indebted to Dr. L. O. Kunkel, Dr. F. O. Holmes, and other members of The Rockefeller Institute for Medical Research, Princeton, N. J., for advice and criticism offered during the investigation reported in this paper and during preparation of the manuscript.

Source of Viruses and Dodder

The viruses used in these experiments were obtained from the collection carried at this Institute. Susceptible plants were first infected mechanically or by grafting with the virus desired. After the inoculated plants showed systemic symptoms, healthy dodder stems were established on the diseased plants and the dodder growth developed on them constituted the source of viruliferous dodder. A constant supply of healthy dodder was kept on tomato or tobacco plants. All experiments were made with *Cuscuta campestris*.

Transfer of Vector

In all cases dodder stems 4–8 inches long were used in making transmissions. This was found more desirable than connecting the test plants to dodder that was still attached to diseased plants. It not only diminished the chances of contamination but also facilitated the handling of individual plants. The dodder stems were always arranged around suitable parts of the host plants, mostly around the younger parts of the stem. The dodder growth was in general left undisturbed on the test plants for about 15 days; then it was pulled off. However, care was taken to prevent connections between adjoining plants, the advancing tips of the dodder stems sometimes being pinched off before the 15 days had elapsed.

Test Plants

Plants of *Lycopersicon esculentum* Mill. var. Bonny Best were used exclusively in the experiments with cranberry false-blossom virus both as sources of inoculum and as test plants. The alfalfa plant, *Medicago sativa* L., served as a plant immune from cranberry false-blossom virus.[3] Tomato and tobacco plants were used in studying tobacco-mosaic and aucuba-mosaic viruses. Crimson clover (*Trifolium incarnatum* L.) and alfalfa were used as plants immune from these viruses. For cucumber-mosaic virus, tobacco and *Nicotiana glutinosa* L. served both as sources of virus and as test plants. In the beginning, soybean, *Glycine max* Merr., was used as a plant immune from the virus (7), but the dodder grew poorly on it. Later trials were, therefore, made with the red clover, *Trifolium pratense* L., also immune from this virus (7).

In most cases dodder was placed on the host plants when they were very young. Not only was it easier to establish the dodder on young plants, but the incubation periods of the diseases were shorter than in old plants.

The development of symptoms was used as a criterion of transmission in the case of cranberry false-blossom virus. With cucumber-mosaic, ordinary tobacco-mosaic, and aucuba-mosaic viruses, judgment as to transmission was based not only on the development of symptoms but also on results of subinoculations to tobacco, *Nicotiana glutinosa, N. sylvestris* Speg. and Comes, or *Vigna sinensis* (L.) Endl. var. Black. The host plants that

[3] Kunkel, L. O. Unpublished work.

showed no symptoms were tested by mechanical inoculation to one or another of these species shortly before being discarded. The immune plants serving as hosts for the dodder also were tested for the virus concerned by subinoculation to one of these susceptible species.

In experiments with cranberry false-blossom virus, the tomato test plants were kept for observation until 90 days after the dodder stems had been established on them. In the cases of tobacco mosaic and aucuba mosaic, the test plants were kept only 40 days, being then tested and discarded. With cucumber-mosaic virus, the test plants were discarded 30 days after they had been parasitized by dodder.

Controls

Healthy dodder stems were placed on the control plants in the case of cranberry false-blossom virus. In the experiments made with viruses mechanically transmissible, control plants were parasitized with viruliferous dodder stems in the same manner as the test plants. A short time after this had been done, the viruliferous dodder was removed and a healthy dodder stem substituted. It was thought that this procedure would afford a check against the possibility of mechanical contamination when placing the dodder on the test plants.

Other details regarding technique will be given with data on the experiments concerned.

EXPERIMENTAL

Negative Evidence on the Multiplication of Ordinary Tobacco-Mosaic and Aucuba-Mosaic Viruses in Dodder

In a study of the multiplication of ordinary tobacco-mosaic and aucuba-mosaic viruses in dodder, an attempt was made first to measure the virus in dodder stems quantitatively by mechanical inoculation. Soon it was learned that the dodder juice had an inhibitory effect on both viruses and that both occurred only at a very low concentration in dodder.

Inhibitory Action of Dodder Juice on Ordinary Tobacco-Mosaic and Aucuba-Mosaic Viruses. Preliminary attempts to recover ordinary tobacco-mosaic virus from dodder stems grown on diseased plants showed that very little virus could be recovered. Three experiments were made. In each, 30 leaves of *Nicotiana glutinosa* and 10 Turkish tobacco plants were rubbed with dodder juice from stems grown on diseased plants. Carborundum was added to the inoculum. A total of 37, 15, and 20 lesions was obtained on *N. glutinosa* and a total of 9, 5, and 4 infected tobacco plants, respectively. Without the addition of carborundum it was difficult to get a single lesion on *N. glutinosa*. Similar results were obtained with aucuba-mosaic virus.

In table 1 results are given of experiments on the inhibitory action of dodder juice on ordinary tobacco-mosaic virus as measured on *Nicotiana glutinosa*. The virus solutions were prepared at 3 concentrations (10^{-1}, 10^{-2}, and 10^{-3}) in water or 0.1 M neutral phosphate buffer. Equal parts

TABLE 1.—*Inhibitory action of dodder juice on tobacco-mosaic virus as measured by the number of local lesions secured on Nicotiana glutinosa. Concentration of dodder juice 1 : 2*

Experiment No.	Dilution, treatment, and average number of lesions[a]					
	$\frac{1}{2} \times 10^{-1}$		$\frac{1}{2} \times 10^{-2}$		$\frac{1}{2} \times 10^{-3}$	
	Dodder juice	Control	Dodder juice	Control	Dodder juice	Control
1[b]	9.9	52.8	2.0	15.2	0.1	7.3
2[b]	15.9	84.6	4.1	43.5	1.0	10.1
3[b]	7.3	23.9	2.6	20.6	0.6	6.5
4[c]	2.1	29.3	0.4	9.8	0.3	4.8
5[c]	4.8	43.5	0.5	22.3	0.1	6.4
Average	8.0	46.8	1.9	22.3	0.4	7.0

[a] Average number of lesions per half-leaf based on 10 half-leaves.
[b] Diluent distilled water.
[c] Diluent 0.1 M phosphate buffer at pH 7.

of virus solutions and normal dodder juice were then mixed shortly before inoculation. Controls were brought to the same dilution by addition of water or buffer, as required. The results show that at the 3 dilutions tested, viz., $\frac{1}{2} \times 10^{-1}$, $\frac{1}{2} \times 10^{-2}$, and $\frac{1}{2} \times 10^{-3}$, the same amount of dodder juice reduced the infectivity of the inoculum by 82.9, 91.5, and 94.3 per cent, respectively. The results suggest that the action of the inhibitor is, in part, on the virus itself (2, 8).

The low concentration of the virus in dodder grown on diseased plants and the inhibitory action of the dodder juice may perhaps explain why Bennett (1) could not recover the virus by mechanical inoculation from *Cuscuta subinclusa* grown on diseased plants.

Experiments on the Transmission of Ordinary Tobacco-Mosaic and Aucuba-Mosaic Viruses by Dodder Taken Directly from Diseased Plants and

TABLE 2.—*Transmission of tobacco-mosaic and aucuba-mosaic viruses by the dodder, Cuscuta campestris, from tomato to tomato*

Virus of	Test plant	Nature of dodder stems used to parasitize the test plants		
		Dodder from diseased plants	Dodder originally grown on diseased plants and then transferred through a series of immune plants	
			After 1st transfer	After 2nd transfer
Tobacco mosaic	*Lycopersicon esculentum* var. Bonny Best	5/31[a]	0/48	0/10
–	Control	0/10	0/20	0/5
Aucuba mosaic	*Lycopersicon esculentum* var. Bonny Best	2/28	0/23	0/19
–	Control	0/10	0/10	0/10

[a] The numerator indicates the number of infected plants and the denominator the number of plants that were parasitized with the viruliferous dodder.

after Transfers Through Immune Plants. Owing to the difficulty of measuring ordinary tobacco-mosaic and aucuba-mosaic viruses in dodder stems by quantitative methods based on local lesions, it was decided to investigate the behavior of these viruses in dodder by making successive transfers of viruliferous dodder stems on immune plants. After each transfer, the dodder growth was tested for virus by mechanical inoculation and by transfer to susceptible host plants. It was thought that these transfers would provide an increasing dilution of the viruses unless they could multiply in dodder.

In tables 2 and 3 are presented the results of transmission of ordinary tobacco-mosaic and aucuba-mosaic viruses through dodder when taken directly from diseased plants and after one and two transfers on immune plants. It will be seen that in a few cases transmission was obtained through dodder taken directly from diseased plants, but that in no case was

TABLE 3.—*Transmission of tobacco-mosaic and aucuba-mosaic viruses by the dodder, Cuscuta campestris, to tobacco plants*

Virus of	Test plant	Dodder stems grown on diseased plants		Control
		From tomato plants	From tobacco plants	
Tobacco mosaic	Turkish tobacco	1/4[a]	1/4	0/4
Aucuba mosaic	" "	1/18	.....	0/9

[a] See footnote, table 2.

transmission obtained after the first or second transfer. The mechanical inoculations confirmed this point. No lesions were obtained on *Nicotiana glutinosa* inoculated with dodder extract taken after the transfers.

These results confirm the view of Johnson (4) that the ordinary tobacco-mosaic virus does not multiply in dodder and show that this is also true for the aucuba-mosaic strain.

Multiplication of Cucumber-Mosaic Virus in the Dodder, Cuscuta campestris

The results obtained with cucumber-mosaic virus in dodder, *Cuscuta campestris,* confirmed the findings of Bennett (1) that cucumber-mosaic virus infects and multiplies in dodder.

Transmission of Cucumber-Mosaic Virus by Viruliferous Dodder Grown on Immune Plants. Dodder stems grown on diseased plants transmitted cucumber-mosaic virus to almost all susceptible plants on which they were placed and established. In one experiment, 7 tobacco plants of 9 that had been parasitized with dodder from diseased plants became affected and 7 of 8 *Nicotiana glutinosa* plants. Four tobacco plants and 7 *N. glutinosa* plants used as controls remained healthy. In another experiment, 16 of 20 Turkish tobacco plants were infected and none of 10 controls. In a third experi-

TABLE 4.—*Transmission of cucumber mosaic virus through dodder taken directly from diseased plants and after transfers on immune plants*

Dodder tested by	Test plant	Nature of the dodder stems				
		From diseased plants	Grown on diseased plants and then transferred on immune plants after			
			1st transfer	2nd transfer	3rd transfer	4th transfer
Parasitizing the test plants	*Nicotiana tabacum* Turkish	4/6[a]	3/4	4/4	4/4	.....
Control	"	0/4	0/4	0/4	0/4	.....
By mechanical inoculation	*N. tabacum*	5/5[b]	5/5	5/5	5/5	5/5
Control	"	0/5	0/5	0/5	0/5	0/5
By mechanical inoculation	*N. glutinosa*	5/5	5/5	5/5	.....	5/5
Control	"	0/5	0/5	0/5	.....	0/5

[a] See footnote, table 2.
[b] Denominator gives the number of plants inoculated and the numerator the number of plants infected.

ment, all of 7 Turkish tobacco plants were infected and none of 5 controls. The incubation period in these experiments varied from 9 to 20 days.

In table 4 are presented some results comparing the transmission of cucumber-mosaic virus through dodder taken directly from the diseased plants and after transfers on immune plants. From the table it can be seen that, even after the 4th transfer, virus was present in dodder at approximately the same concentration as in dodder grown on diseased plants. Calculations showed that the 4th transfer would correspond to a dilution of about 1:5,000,000 of the initial virus concentration in the dodder. This is beyond the dilution end point of the virus. During the transfers the virus was retained in dodder for 4 months.

Mechanical Inoculation of Dodder Stems with Cucumber-Mosaic Virus. Additional evidence that cucumber-mosaic virus infects dodder and multi-

TABLE 5.—*Mechanical inoculation of the dodder, Cuscuta campestris, with cucumber-mosaic virus*

Experiment No.	Test plant and number of dodder stems out of the total inoculated that transmitted the virus			
	Nicotiana tabacum		*Nicotiana glutinosa*	
	Dodder inoculated mechanically	Control	Dodder inoculated mechanically	Control
1	4/8[a]	0/4	3/8	0/4
2	1/8	0/4	0/8	0/4
3	2/14	0/8	.....	.....
Total	7/30	0/16	3/16	0/8

[a] The denominator indicates the number of dodder stems that were inoculated and the numerator the number that transmitted the virus to the test plants.

plies in it was obtained by mechanical inoculation of dodder stems. This was done by rubbing vigorous dodder stems 4–8 inches in length, along $\frac{2}{3}$ of the basal parts, with a small cotton swab dipped in the inoculum to which carborundum had been added. Undiluted juice or juice diluted 1:5 with 0.1 M neutral phosphate buffer was used. The rubbing was not done near the tips of the dodder stems because it had been found that, when the tips were rubbed, it was difficult to obtain establishment of the dodder on host plants. After inoculation the stems were rinsed in water and placed on susceptible plants and also on immune plants. In table 5 are presented the results obtained when the inoculated dodder was placed on Turkish tobacco or on *Nicotiana glutinosa*. Table 6 gives the results when the inoculated dodder was placed on red clover plants and then tested for virus at different intervals of time. In a few cases the inoculated dodder stems transmitted the virus to the susceptible plants on which they were placed or virus

TABLE 6.—*Number of dodder stems inoculated mechanically with the cucumber-mosaic virus and from which the virus was recovered after they had been established on red clover, Trifolium pratense. Recovery by mechanical inoculation to tobacco and Nicotiana glutinosa plants*

Experiment No.	Extract from inoculated dodder grown on immune plants. Tested after		
	1 day	7 days	15 days
1	0/10[a]	.....	4/10
2	0/10	2/10	2/20
3	0/10	0/20	0/20
Total	0/30	2/30	6/50

[a] The denominator indicates the number of inoculated dodder stems that were tested and the numerator the number that contained the virus as tested by mechanical inoculation.

was recovered from them after they had been established on immune plants. The evidence indicates that the virus infected the dodder and multiplied in it. When inoculated dodder was placed on susceptible plants, it could be conceived that the virus mechanically introduced into the dodder might pass to the test plant and cause infection. However, this would not be possible when the dodder was placed on red clover plants that were immune from the virus. The clover plants were tested for virus with negative results.

Local Lesions Caused by Viruliferous Dodder at the Point of Attachment. In preliminary experiments, dodder stems containing cucumber-mosaic virus established on *Vicia faba* L. plants caused the appearance of black elongated necrotic lesions at the point of attachment. Control plants on which healthy dodder had been established showed no such lesions. In another experiment, local lesions were observed in 9 of 10 plants on which viruliferous dodder stems were placed and in none of 3 controls. The local lesions were visible from 5 to 7 days after the dodder had been placed on the test plants. In a third experiment, necrosis near the point of attachment

began to show on the 4th day after the dodder had been placed on the plants. Leaves of *V. faba,* when inoculated by rubbing with the cucumber-mosaic virus, show necrotic local lesions in about 2 or 3 days. The lesions near the point of attachment of the dodder may appear in 4 days. This is a very short time if we consider that it takes 1 or 2 days for the dodder to make a haustorial connection with the host plant. It also shows that infection occurs almost immediately after organic union between dodder and host plant takes place and that virus is present in the cells involved in the process of haustoria formation.

Local lesions near the point of attachment also were obtained when viruliferous dodder stems were placed on *Vigna sinensis.* The lesions were reddish but not very conspicuous.

Recovery of Cucumber-Mosaic Virus from Dodder Stems and Inhibitory Effect of Dodder Juice. Preliminary attempts to recover the virus from

TABLE 7.—*Inhibitory effect of dodder juice on the cucumber-mosaic virus as measured by the number of local lesions secured on Vigna sinensis var. Black. Virus diluted to 1:5. Diluent 0.1 M neutral phosphate buffer*

Experiment No.	Concentration of dodder juice and average number of lesions[a]					
	0	3:5	2:5	1:5	1:25	1:125
1	15.5	0	0	0	.....	.....
2	37.0	0	0	0	.....	.....
3	15.6	.....	.....	.....	0.4	2.1
4	39.8	.....	.....	.....	0.2	3.6

[a] Average number of lesions per leaf on 30 leaves.

dodder stems grown on diseased plants showed that, although some virus was present, it was difficult to demonstrate this by inoculation to *Vigna sinensis,* in which the virus produces local lesions. Virus could with some difficulty be recovered by rubbing the dodder juice onto tobacco plants. Virus could also be demonstrated in dodder by placing it on host plants that would become infected.

Some trials were made by mixing cucumber-mosaic virus inoculum with different amounts of dodder juice. In one trial the virus concentration was kept constant at 1:5 and the dodder juice was tried at 3 concentrations: 1:5, 2:5, and 3:5. Each concentration of inhibitor was inoculated on 30 leaves of *Vigna sinensis,* the opposite leaves being inoculated with a control preparation containing no dodder juice. Carborundum was always used for inoculation. The results presented in table 7 show that at 1:5 the dodder juice completely inhibited the infectivity of the inoculum as tested on *V. sinensis.* A second experiment gave identical results. A 3rd and 4th experiment were then made by adding 1 cc. of dodder juice at various concentrations to a mixture of 1 cc. cucumber-mosaic virus extract + 3 cc. of 0.1 M phosphate buffer at pH 7. Two concentrations of dodder juice were used, 1:5 and 1:25. The final concentration of virus after mixture

with dodder juice was 1:5 and of dodder juice 1:25, 1:125, and control. The results show that, even at 1:125, the dodder juice had a pronounced inhibitory action. The data obtained do not permit a statement as to whether the inhibitory action is on the virus or on the host plant.

Symptoms Exhibited by Dodder Infected with Cucumber-Mosaic Virus. Apparently, dodder stems infected with cucumber-mosaic virus show some symptoms of disease. This was observed as an almost constant feature in the experiments, and, whereas no certainty exists as to its nature, it seems worthy of being reported.

The symptoms consist of a distorted type of growth. This is illustrated in figure 1. The degree of distortion may vary, but it can always be per-

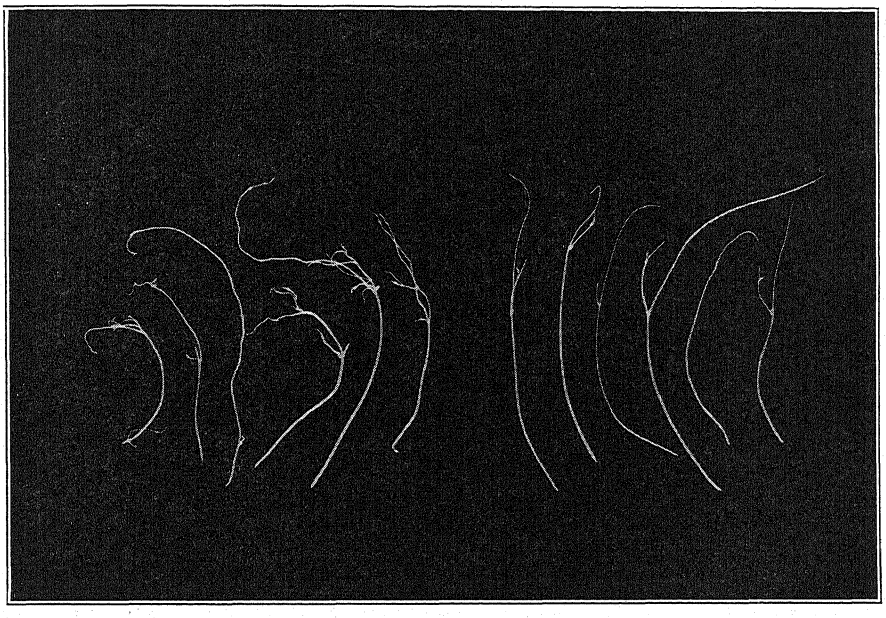


FIG. 1. The distorted type of growth exhibited by dodder stems infected with cucumber-mosaic virus. Healthy dodder stems at the right. (Photograph by J. A. Carlile.)

ceived. The distorted type of growth could, in most instances, be noticed after the viruliferous dodder had been transferred on a series of immune plants. In addition, the growth of viruliferous stems was apparently slower than normal. It was also found much more difficult to establish the dodder on a plant affected by cucumber mosaic than on plants affected by tobacco mosaic or on healthy controls.

The distortion above described may occasionally be brought about by adverse conditions. There may remain some doubt, therefore, whether the observed symptoms are really the result of the virus. In no case, however, were these symptoms found associated with tobacco-mosaic virus or cranberry false-blossom virus.

Multiplication of Cranberry False-Blossom Virus in the Dodder, Cuscuta campestris

The multiplication of cranberry false-blossom virus in dodder was studied by successive transfers of viruliferous dodder on a series of immune alfalfa plants. By this means an increasing dilution of the virus present in dodder took place. Six transfers were made in one experiment and 5 transfers in each of 4 other experiments. The calculations showed that each transfer would correspond to at least a tenfold dilution. After each transfer, samples of dodder stems were placed on tomato test plants and permitted to become established in the manner already described. Table 8 records the results obtained.

TABLE 8—*Transmission of cranberry false-blossom virus by the dodder, Cuscuta campestris. Test plant, tomato. Immune plant, alfalfa*

Experiment	Nature of the dodder stems used to parasitize the test plants						
	From diseased plant	Grown on diseased plants and then transferred on immune plants after					
		1st transfer	2nd transfer	3rd transfer	4th transfer	5th transfer	6th transfer
1	18/23[a]	27/31	10/18	9/19	7/21	0/24	5/38
2	15/20	9/14	11/15	19/27	20/26	9/23	.....
3	8/10	12/15	4/15	5/9	3/11	12/27	.....
4	13/20	11/16	10/18	11/29	15/22	3/16	.....
5	15/20	13/16	8/16	8/16	9/25	4/19	.....
Total	69/93	72/92	43/82	52/100	52/105	27/109	5/38
Control	0/25	0/25	0/23	0/20	0/22	0/25	0/5

[a] See footnote, table 2.

As can be seen from the table, there was a decrease in the percentage of infected plants after the transfers, but this decrease was not marked and apparently was not due to dilution. Two possible explanations may account for this. First, it may be said that the decrease was due to environmental conditions. The first transfers were performed in winter when the greenhouse temperature was kept around 75° F. The last transfers, on the other hand, were made in summer when the greenhouse temperature sometimes goes very high. Kunkel (5) has already shown that cranberry false-blossom virus is sensitive to heat. The second explanation is that when the dodder stem is taken from a diseased plant, the virus titer may be higher than after transfer to an immune plant. This is easy to understand because, in addition to the virus infecting the dodder, some virus might be absorbed from the diseased plant along with food.

It is interesting to note that, in addition to withstanding a dilution of at least 10^{-6}, the virus was retained in dodder for about 100 days. Considering these facts, it seems reasonable to conclude that cranberry false-blossom virus multiplies in dodder.

DISCUSSION

Tobacco-mosaic and aucuba-mosaic viruses, although highly infectious, are transmitted through dodder taken from diseased plants in only a small percentage of cases and not through dodder from diseased plants after 1 or 2 transfers on immune plants. This constitutes evidence that these viruses do not multiply in dodder. The period of incubation (longer than in mechanical transmission) also confirms this point and suggests that transmission is dependent upon the movement of materials through the dodder rather than upon close contact of infected dodder tissue with the host plant.

Cucumber-mosaic virus shows a different behavior in its transmission through dodder. The virus is easily transmitted through viruliferous dodder. The incubation period is relatively short, the majority of plants showing symptoms from 9 to 12 days after the viruliferous dodder stems are placed on them. Moreover, the viruliferous dodder retained the virus after 4 successive transfers on immune hosts made during a period of 4 months, without an apparent decrease in virus concentration. Experiments with *Vicia faba,* on which the viruliferous dodder causes local lesions near the point of attachment, showed that passage of virus from the dodder to the host plant occurs probably at the time of haustoria formation. This suggests that the virus becomes systemic in dodder and that the cells involved in haustoria formation contain it. Dodder stems could also be inoculated mechanically with cucumber-mosaic virus. This, too, is evidence that the virus is able to infect the dodder and multiply in it. The observation that certain symptoms of disease persisted in dodder stems that had been grown on diseased plants and transferred to immune plants also supports the view that this virus infects the dodder. Additional evidence as to the infection of dodder stems by cucumber-mosaic virus could perhaps be obtained by protection tests. Infection of dodder with cucumber-mosaic virus by aphids would also furnish additional evidence on multiplication.

The cranberry false-blossom virus could be recovered from viruliferous dodder after 6 successive transfers on immune plants made during a period of 4 months. This corresponds to a dilution of at least 10^{-6}. The evidence is highly suggestive that the virus multiplies in the dodder. The number of plants infected by viruliferous dodder is also relatively high.

SUMMARY

The ordinary tobacco-mosaic and aucuba-mosaic viruses were transmitted through the dodder, *Cuscuta campestris,* in a few cases when the dodder was taken directly from diseased plants. The incubation period varied from 12 to 20 days. No case of transmission was obtained after dodder from diseased plants had been transferred once or twice through alfalfa or crimson clover plants immune from the virus.

Dodder juice has an inhibitory effect on ordinary tobacco-mosaic and aucuba-mosaic viruses as measured by the number of local lesions secured on *Nicotiana glutinosa.* Apparently the action is, in part, on the virus.

High percentage of transmission through dodder was obtained with cucumber-mosaic virus. Viruliferous dodder stems, grown after 4 successive transfers through red clover immune from the virus, showed no decrease in virus content. These 4 transfers would correspond to a dilution beyond the dilution end point of the virus. The incubation period varied from 9 to 20 days, most plants showing symptoms in from 9 to 12 days.

Local lesions near the point of attachment were obtained by placing dodder stems containing cucumber-mosaic virus on *Vicia faba*. The lesions appeared in 4 to 5 days.

Dodder juice also had an inhibitory effect on cucumber-mosaic virus as measured on *Vigna sinensis* var. Black.

Dodder stems were successfully inoculated by rubbing with cucumber-mosaic virus. This was shown by placing the dodder on test plants or by establishing it on immune plants and then recovering the virus by inoculation.

Dodder stems infected by cucumber-mosaic virus showed in most cases a distorted type of growth. The degree of distortion varied, but it could always be perceived.

Cranberry false-blossom virus was retained in viruliferous dodder after 6 successive transfers on alfalfa, which is immune from this virus.

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ROOT INFECTION OF CROP PLANTS AND WEEDS BY TOBACCO LEAF-SPOT BACTERIA[1]

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Angular leaf spot and wildfire, caused by *Bacterium angulatum* F. and M. and *Bact. tabacum* W. and F., respectively, have caused loss in plant beds and in the field in Kentucky for many years. In burley plant beds angular leaf spot is almost certain to develop, when the plants are about ¼ grown, during a wet, cool period. It is rarely conspicuous in dark tobacco beds. During exceptionally wet springs, wildfire may break out in areas where it had not been observed before. For example, in the spring of 1943 wildfire could be found in nearly half of the untreated beds in Central Kentucky in areas where it was almost unknown before. No satisfactory explanation has been given for outbreaks of either disease in plant beds until recently. The explanations that the bacteria are seed-borne; that they are blown into beds on bits of infected tobacco trash; or that other plants that have become water-soaked and infected, harbor the bacteria for months, and then act as a source of infection, have not been adequate to explain general infection of tobacco beds following a wet period.

Another feature of the diseases that has needed explanation is that both diseases can be completely controlled in plant beds, as they are handled in Kentucky, if 2 applications of 3–4–50 Bordeaux are made to the surface of the soil when the plants are small. The only purpose of the second application appears to be to make certain that spots that may have been missed the first time are treated. The treatments are not designed to cover the leaves because, at the time of application, leaves that would later be susceptible have not yet developed. The purpose has been to coat thoroughly the surface of the soil with Bordeaux.

Recently, in Science,[2] we reported that both *Bacterium angulatum* and *Bact. tabacum* live on the roots of various crop plants and can be perpetuated in the field from one season to another by this means. The present paper presents evidence on this phase of the problem and its relation to outbreaks of the diseases in the plant bed and the field.

CARRY-OVER IN SOIL

Clinton and McCormick[3] demonstrated that collections of soil made in March and April, in a garden plot where wildfire-affected tobacco had grown the year before and allowed to rot in place, were capable of causing infection on pricked tobacco leaves in the greenhouse. Later studies by others did not

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Valleau, W. D., E. M. Johnson, and S. Diachun. Association of tobacco leaf-spot bacteria with roots of crop plants. Science 96: 2485, p. 164. 1942.

[3] Clinton, George P., and Florence A. McCormick. Wildfire of tobacco in Connecticut. Conn. Agr. Exp. Stat. Bull. 239. 1922.

confirm these results, and the tendency was toward not accepting the possibility of overwintering in moist soil. Wolf,[4] in 1922, found that some beds in which wildfire was present one year, if used the second year, sometimes developed wildfire when clean seed and new cotton were used. He believed this was evidence in favor of overwintering in soil. An abundance of the same kind of evidence in favor of general soil infestation can be obtained each year in areas where plant beds are placed in a new site and clean cotton is used. Wildfire and angular leaf spot are as likely to develop in these new sites as in old ones.

Positive proof of overwintering of *Bacterium angulatum* in soil was presented in abstract in 1942 by the present writers.[5] Using an improved technique for isolation of pathogenic bacteria from the soil, which consisted in watersoaking a leaf[6] and then pouring the soil to be tested, thoroughly stirred in an excess of water, over the undersurface, it was possible to show that the bacteria overwintered in considerable numbers in field soils in which crops infected with angular leaf spot were grown the previous summer. In these tests, extending over 2 winters, 10 or 12 soil samples, each consisting of slightly less than ½ tumbler of soil, were collected each time from a plot and tested on separate tobacco leaves. In 1940–41, 182 samples were collected between November 2 and April 17 on 15 different days from a plot on which tobacco had been heavily infected with *Bacterium angulatum* the summer of 1940 and planted with rye in the fall. Thirty-seven soil samples collected on 9 different occasions caused angular leaf spot. The number of spots ranged from 2 to 300 per leaf. Seventy-two samples collected on 6 dates between May 8 and July 16, 1941, from the same plot all failed to give infection. All of this period was after the cover crop had been plowed under. The following winter the tests were repeated in two plots that had been in continuous tobacco for several years and planted with a rye cover crop during the winter. Angular leaf spot and wildfire were present in the tobacco in 1941 in the first plot and only angular leaf spot in the second. The results obtained were similar to those of the previous year. From the first plot 160 samples were collected on 16 days between August 27, 1941, and March 24, 1942. Of these, 56, or 35 per cent, gave angular leaf spot or wildfire or both. There were a total of 659 angular leaf spots and 14 wildfire spots. The number of spots per leaf ranged from 3 to 250. Sixty collections made between April 13 and June 20, 1942, gave no infection.

From the second plot 180 collections were made between August 27, 1941, and April 21, 1942. Of these, 51, or 28 per cent, gave angular leafspot. A total of about 2023 spots were produced, ranging in number between 1 and

[4] Wolf, F. A. Wildfire of tobacco. N. C. Agr. Exp. Stat. Bull. 246. 1922.

[5] Diachun, Stephen, W. D. Valleau, and E. M. Johnson. Isolation of *Bacterium angulatum* from overwintered tobacco field soil. Phytopath. 32: 2–3. 1942.

[6] Water soaking is done by directing a stream of water preferably against the lower side of the leaf when the stomata are open. In the present study, it was done with a 10- or a 40-cc. injection syringe filled with water and the needle held about an inch from the leaf surface. If the stomata are open, the tissues water-soak quickly and water soaking disappears within about 15 minutes, leaving no sign of injury.

1700 on individual leaves. These tests afforded positive proof that both *Bacterium angulatum* and *Bact. tabacum* survive in field soil over winter, but give no suggestion as to the means of survival in the soil.

CARRY-OVER IN ASSOCIATION WITH PLANT ROOTS

With the idea in mind that certain cover crops might tend to depress the overwintering of the organisms, 6 small plots were laid out, of which 5 were inoculated on October 11, 1941, with cultures of *Bacterium angulatum* and one with crushed dried leaves infected with angular leaf spot. Three plots were seeded with crimson clover, vetch, and wheat, respectively, and 3 left fallow.

TABLE 1.—*Number of angular leaf spots produced by pouring upon a water-soaked tobacco leaf a mixture, with water, of 10 small soil samples from a plot. The plots were inoculated October 11, 1941, as indicated*

When tested	Plots with cover crops			Fallow plots		
	Crimson clover	Hairy vetch	Winter wheat			
	Soil inoculated with					
	2 pure cultures				Mixed cultures	Infected dried tobacco leaves
1941						
Oct. 22	600	400	600	400	400	300
Nov. 14	6	19	67	2	23	0
Dec. 6	17	116	50	1	1	500
Dec. 15	7	15	39	300	0	0
Dec. 23	45	6	146	2	0	2
1942						
Jan. 13	14	69	5	0	0	0
Jan. 22	31	69	16	0	0	0
Feb. 5	66	6	500	1	0	11
Feb. 12	80	180	180	16	9	0
Feb. 19	108	54	60	3	5	53
Mar. 4	31	18	74	0	5	0
Mar. 17	50	72	12	0	0	0
Apr. 2	30	14	11	0	0	0
Apr. 20	33	320	23	0	3	0
May 5	11	9	4	0	0	0
May 23	0	0	0	0	0	0
Totals	1189	1367	1787	725	446	866

On 16 different dates between October 22, 1941, and May 23, 1942, a composite of 10 small samples was taken from each plot and tested together on water-soaked leaves. The results of these inoculations are given in table 1. On every date except the last, *Bacterium angulatum* was isolated in the inoculated tobacco leaves. It is evident from the table that the organism was isolated much more consistently from the plots with cover crops (45 times) than from the fallow plots (20 times). These results suggested, during the winter, the possibility that the organisms were growing on the roots of the crop plants and perhaps also on the roots of occasional weeds that were pres-

TABLE 2.—*Results of inoculation with washed roots of crop plants and weeds grown in soil inoculated with Bacterium angulatum November 11, 1941. Roots collected at intervals between February 19, 1942, and June 4, 1942*

	Crimson clover	Vetch	Wheat	Henbit	Chick-weed	Oxalis	Carda-mine
Number of tests	9	9	10	6	3	2	1
Number positive	6	8	8	6	1	0	0
Total roots tested	45	45	46	23	7	8	2
Number positive	18	22	33	9	2	0	0
Range of number of spots on inoculated leaves	1 to 56	1 to 300	3 to 800	1 to 40	4 to 14	...	...

ent in the fallow plots. Therefore, tests to determine this point were conducted with the roots of plants growing in these plots.

Roots to be tested were dug on 5 different days between February 19, 1942, and June 4, 1942. The roots were washed in running water, and dirt particles were gently rubbed from them with a bit of paper towelling. The roots were then crushed in water and the water poured over the under surface of a water-soaked leaf. Usually, 5 separate root systems of each species were tested on each date. Heavy infection resulted from some of the roots of each of the crop plants in all tests except those made on June 4, when slight infection was obtained from vetch only. At this time the crimson clover and wheat plants were dried and the vetch nearly dead. A summary of these tests is given in table 2. The results indicate that during the winter and spring *Bacterium angulatum* is closely associated with the roots of several plants unrelated to tobacco.

Collections of roots of rye were made from 2 plots on the station farm, in which tobacco was affected with angular leaf spot in 1941, to determine whether roots of a cover crop following tobacco would harbor *Bacterium angulatum* over winter. Collections were made March 11 and 23 and April 7, taking 20 roots on each date from each plot sampled. These were carefully washed as before in running water, crushed, and inoculations made.

The results of inoculations presented in table 3 give proof that *Bacterium angulatum* is maintained overwinter in close association with the roots of rye planted following an infected tobacco crop.

The preceding tests dealt primarily with *Bacterium angulatum*. *Bact. tabacum* appears to live in association with roots in the same way, as indi-

TABLE 3.—*Inoculations from washed roots of rye collected in March and April in a field in which tobacco was naturally infected with angular leaf spot the previous summer*

	Plot 1	Plot 2
Number of tests	3	2
Number of tests positive	3	2
Number of roots tested	60	40
Number of roots positive	12	13
Range in number of spots	1 to 80	1 to 51

cated by the results of inoculations made with washed rye roots collected during late winter from a field in which wildfire was present in tobacco the previous summer. Collections of soil and roots were made March 27, April 15, May 5, and June 1, 1942. *Bact. tabacum* was isolated in tobacco leaves on each occasion, but less frequently as the plants became older. On March 27, 80 roots, and 110 small soil samples, with no attention to whether they contained roots or not, were collected. The soil clinging to the roots was knocked off, making a composite soil sample from every 10 roots. The roots were washed as previously described, and 10 root systems crushed together. Inoculations were then made on water-soaked leaves with soil, soil from roots, and crushed roots. The results presented in table 4 show that *Bact. tabacum* can be isolated from soil samples (which may have contained roots), from soil shaken from roots, but was obtained in greatest abundance from the washed roots. It seems evident that *Bact. tabacum,* as well as *Bact. angulatum,* can live in association with the roots of rye over winter.

A similar test was made with barley roots collected from a field in which angular leaf spot was present the previous summer, and with wheat roots from a field in which both wildfire and angular leaf spot were present the previous summer. The barley roots caused extensive angular leaf spot and the wheat roots caused mixed infection of angular leaf spot and wildfire, on inoculated tobacco leaves.

TABLE 4.—*Inoculations from soil, soil shaken from roots and crushed rye roots collected March 27, 1942, from a field infected with wildfire in 1941*

No. of tests and wildfire spots	Soil	Soil from roots	Roots
Number of tests	11	8	8
Number of tests positive for wildfire	2	8	8
Total number of wildfire spots	2	57	222

CARRY-OVER THROUGH SECOND WINTER IN ABSENCE OF TOBACCO

In the spring of 1943 soil collections containing crimson clover roots were made in a field that was in tobacco in 1941, wheat in 1941–42, and crimson clover in 1942–43. On April 7 and 8, 1943, 15 collections of 5 cores each were used as inoculum. Fourteen of the 15 caused angular leaf spot, with spots ranging from 6 to 500 on inoculated leaves. These results indicate that the bacteria may be found on the roots of cover-crop plants which do not directly follow tobacco in the rotation.

INFECTION FROM WASHED ROOTS OF TOBACCO FROM PLANT BEDS AND FIELD

Infection in plant beds usually does not appear until the plants are well established and are covering the ground. Then it develops only following a protracted wet, cool period. In view of the association found between the bacteria and cover crops during the winter, it seemed probable that the bacteria in the plant bed might first multiply on the roots of tobacco plants be-

fore causing leaf infections. Therefore, plants were collected from 81 plant beds between April 28 and June 2, 1942. The roots from each bed were washed separately, crushed, and used as inoculum on water-soaked leaves. Angular leaf spot resulted from 22 collections, and wildfire from 4. Infections per inoculated leaf ran as high as 2000. Seven of the beds from which root collections caused angular leaf spot appeared to be entirely free from leaf spots at the time the collections were made.

During the summer of 1942 tobacco roots were collected from the field at intervals between June 18 and September 23. The roots were washed and crushed, and water-soaked leaves inoculated. Fifty-seven roots were collected from plants affected with wildfire, of which 42 produced wildfire on inoculated plants. Of 56 roots from plants affected with angular leaf spot, 2 caused wildfire and 44 angular leaf spot. Thirty-eight roots from plants affected with both caused wildfire on 25 inoculated leaves and angular leaf spot on 17. Sixteen plants were collected that appeared to be free from infection, but were in fields where diseased plants could be found. One caused wildfire and 12 angular leaf spot. These tests demonstrate that both organisms are in close association with the roots of tobacco plants in the plant bed and throughout the summer in the field, until the time for sowing cover crops.

PRESENCE OF BACTERIUM ANGULATUM AND BACT. TABACUM IN PROSPECTIVE PLANT BED SITES

The evidence given proves that *Bacterium angulatum* and *Bact. tabacum* can maintain themselves on the roots of tobacco and several crop plants. It would appear therefore that there is no necessary relation between tobacco and the perpetuation of these organisms in a field. Observational evidence in the past has demonstrated that *Bact. angulatum* is nearly always present in burley tobacco plant beds and *Bact. tabacum* sometimes present if weather conditions are such as to make their presence apparent. It, therefore, seemed likely that the organisms could be isolated from old pastures and fence rows, such as are commonly used for making tobacco beds. Tobacco beds in Fayette and neighboring counties usually are placed in a new site each year, although a few farmers set aside a small lot and rotate the beds with bluegrass sod.

Soil collections were made from the sod in the vicinity of recently prepared beds and from fields that had been in pasture for years and in which beds had not been prepared. A soil sample consisted of 5 cores collected with a soil-sampling tube. The core necessarily contained roots of any plants growing at the point of sampling. Usually 10 such samples were collected from a field, making a total of 50 cores. The samples were brought to the greenhouse, stirred in water until the soil particles had separated, and were then poured over a water-soaked tobacco leaf. Collections were made on 38 farms. No infection resulted from 180 samples from 18 farms, but, from the other 20, angular leaf spot resulted from 16 collections of a

total of 200 tested (Fig. 1, A), wildfire from only 2, and wildfire and angular leaf spot from 3.

One field was of particular interest. The bed was located about 4 feet from the fence and was heavily infected with wildfire throughout its length. The remainder of the field had been plowed and prepared for tobacco. It had been in orchard grass-Korean lespedeza pasture for the past 5 years, following a crop of tobacco. Soil cores were collected along the fence row and in the field, and weeds and seedling lespedeza plants were collected in the field away from the bed and on higher ground. Of the 10 composite sam-

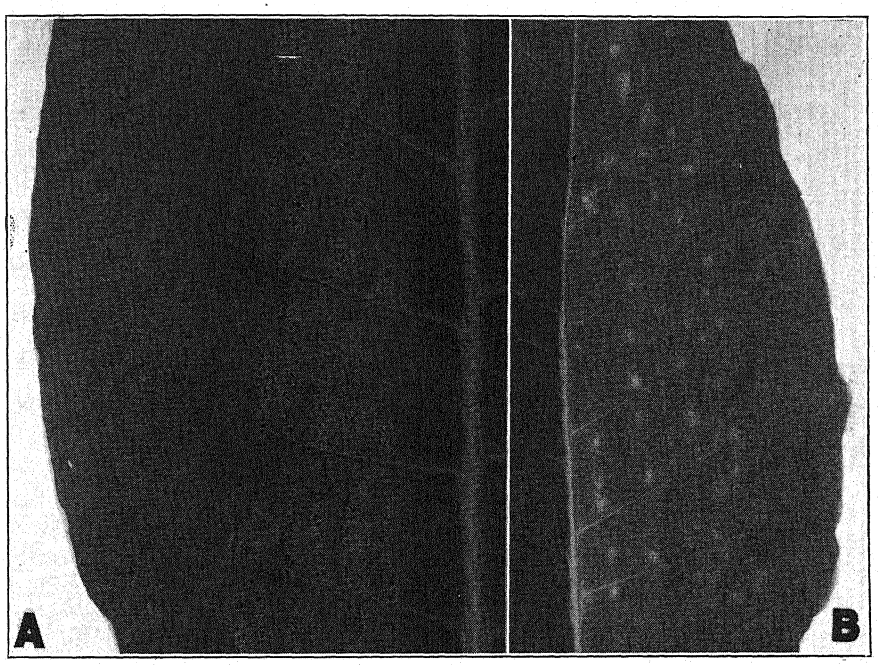


FIG. 1. A. Angular leaf spot resulting from a culture made from a leaf inoculated with field soil containing weed roots. About ½ natural size. B. Wildfire resulting from inoculation of a tobacco leaf with a composite of 5 soil cores collected along a fence row near a wildfire-infested plant bed. About ½ natural size.

ples of soil collected along the fence row, 9 gave wildfire spots ranging in number from 1 to 75 (Fig. 1, B). Two of these caused angular leaf spot also, and 1 sample caused only angular leaf spot. Ten composite soil samples, collected in the plowed field from spots with weeds, gave wildfire alone from 2, wildfire and angular leaf spot from 5, and angular leaf spot from 1. Weeds were dug from the field and fence row, roots were washed, and species tested separately. Angular leaf spot was obtained from roots of ragweed, giant ragweed, white clover, vermifuge and oxalis, and wildfire from roots of Korean lespedeza, shepherd's-purse, white clover, ragweed, chickweed, and orchard grass. At the time these collections were made, some growers were beginning already to set tobacco.

These results prove that *Bacterium tabacum* and *Bact. angulatum* are present in the soil in pastures at the time plants become infected in the plant bed, and that they also are present in association with plant roots in fields plowed and ready to set to tobacco.

COLONIES ON ROOTS

From the evidence presented, it appears that the pathogenic leaf-spot organisms are closely associated with the roots of tobacco, other crop plants,

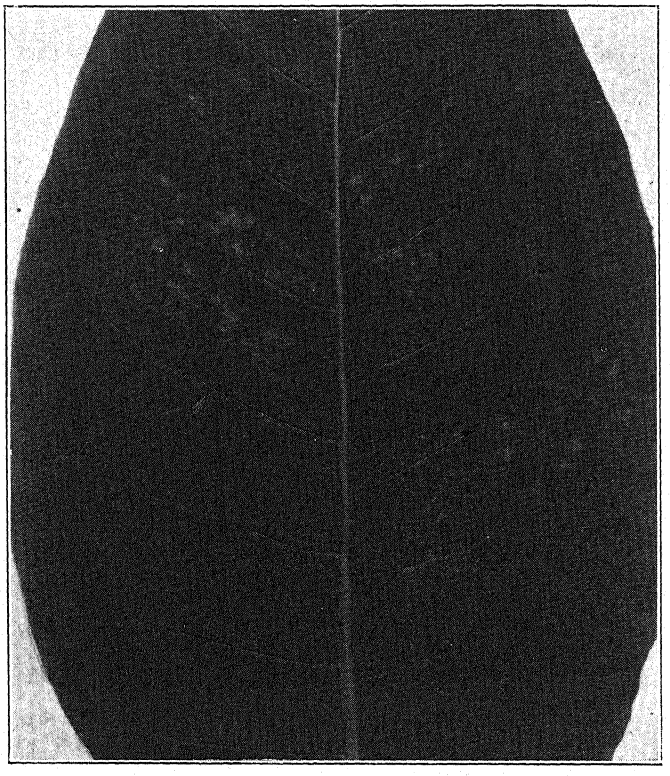


FIG. 2. Wildfire on a tobacco leaf resulting from inoculation with 4 bits of tobacco roots, each bearing a bacterial colony. Each bit of root was crushed separately in water and poured over the under surface of two water-soaked interveinal spaces.

and some weeds. If tobacco roots are removed from a plant bed in an area where plants are injured by either wildfire or angular leaf spot, or if roots are taken from plants in a field affected with one or the other of these diseases and the small rootlets washed and examined with a microscope at low power, colonies of bacteria can be found growing on the surface of the rootlets. Some of the colonies appear soft and watery because motile bacteria can be seen swimming from the surface, while other colonies appear firm, with the surface bacteria remaining in place. To determine whether or not these were colonies of pathogenic bacteria, a very small bit of root bearing a colony was removed, crushed in water, and used as inoculum on a small

water-soaked area of a leaf. When the roots were from plants affected with angular leaf spot, root colonies produced angular leaf spot, and, when from plants with wildfire, this disease resulted from inoculation with root colonies. In all, 59 colonies were selected for test from roots of plants from a tobacco

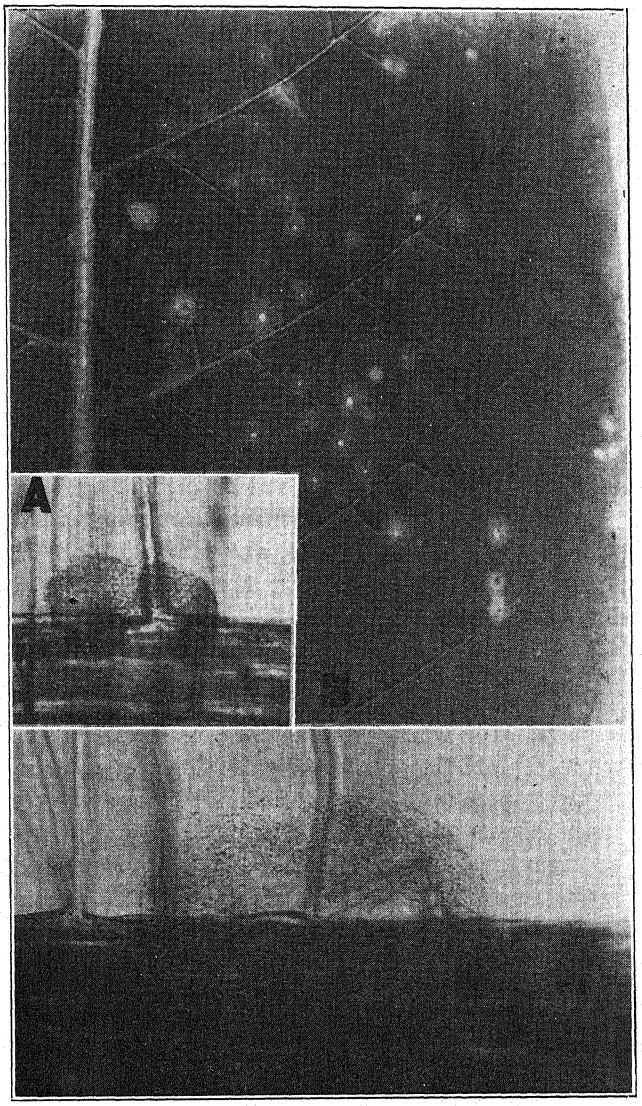


FIG. 3. A. A colony of *Bacterium tabacum* growing on the surface of a wheat rootlet. About ×200. B. Infection produced on a tobacco leaf using colony shown in A as inoculum. C. A colony of *Bact. angulatum* on the surface of a wheat rootlet. About ×200.

bed with wildfire. Fifty of the colonies caused wildfire, with an average of 57 wildfire spots for each inoculation (range 1 to 300) (Fig. 2). Fifty-three colonies from roots of plants from a bed affected with anglar leaf spot were

tested. Forty-six caused angular leaf spot, with an average of 46 spots per leaf (range 2 to 200).

Wheat had proved to be a crop on the roots of which both species of bacteria overwinter. As the seeds are readily surface-sterilized (2 hours in $1\frac{1}{4}$ per cent solution of NaClO), and germinate quickly, wheat was used in further studies of the colonies on roots. Treated wheat seeds were germinated in a damp chamber. When the roots were developed, they were inoculated by dipping into an aqueous suspension of *Bacterium tabacum* or *Bact. angulatum* containing 1 to 2 million cells per cc. After inoculation, the plants were placed on sterile moist paper toweling in Petri dishes. In from 2 to 5 days, colonies could be found on the roots, usually in the region of the root hairs. The colonies were sometimes tenacious and persisted on the roots even after being mounted in water and left for more than 24 hours (Fig. 3, A and C). A total of 96 wheat roots were inoculated with *Bact. tabacum*. Colonies were found on 85 of them. Forty root systems bearing colonies were used as inoculum, and all produced typical wildfire spots on inoculated tobacco leaves. On 66 noninoculated root systems examined, only 1 colony was found. This was obviously a contamination. Twenty noninoculated root systems failed to cause wildfire when used as inoculum.

When individual colonies were removed on a short bit of a wheat root inoculated with *Bacterium angulatum* or *Bact. tabacum,* crushed in water and used as inoculum on tobacco leaves, angular leaf spot or wildfire developed, depending on the organism being tested (Fig. 3, B). These tests leave little doubt that the colonies observed on the wheat roots were those of the two pathogens.

DISCUSSION

In the early studies on wildfire and angular leaf spot, various hypotheses were advanced to explain the overwintering of the organisms and plantbed infection. Most of these were such as would be suggested by a superficial knowledge of the diseases and were not based on actual knowledge of the habits of the organisms. Recently, Reid and co-workers[7] advanced the hypothesis that because the common nonpathogenic *Pseudomonas fluorescens,* and the pathogenic *Bacterium angulatum* and *Bact. tabacum* show antigenic identity, and because *P. fluorescens* could be found on the leaves of common crop plants and in all tobacco bed soils, this organism was a potential danger to tobacco. They state "the authors consider that serological techniques measure the sum of the morphological and physiological characteristics (including pathogenicity) of the germ plasm of bacterial cells." It probably would be generally admitted that serological tests could be used to prove the identity of certain chemical compounds in two species of organisms, but the assumption that antigenic identity proves genetic identity of bacterial cells is one that would be difficult to substantiate. No proof, other

[7] Reid, J. J., J. Naghski, M. A. Farrell, and D. E. Haley. Bacterial leaf spots of Pennsylvania tobacco. I. Occurrence and nature of the microorganism associated with wildfire. Penn. Agr. Exp. Stat. Bull. 422. 1942.

than serological tests, was presented by these authors to support their assumption of identity.

The present writers give evidence to show that the tobacco leaf-spot phase of the life cycle of these two parasitic organisms is more or less accidental and is probably not essential to their perpetuation. They are not, therefore, primarily tobacco pathogens, but are organisms apparently adapted to a life on the surface of small rootlets of several plants, both weed and crop. It has been shown that the organisms are present on the roots of weeds and pasture plants at the time tobacco plants are developing in the bed, that they are present on the roots of tobacco plants in the bed, that they may be isolated from the roots of tobacco plants growing in the field throughout the summer, and that they are present on the roots of cover crops, sowed following harvest, throughout the winter and until the roots die the following spring.

If, during the period the plants are in either bed or field, the leaves become water-soaked, either by the internal process or from outside causes such as driving rain, and the pathogenic bacteria are splashed or otherwise carried to the water-soaked areas, the bacteria enter immediately, colonies develop, and leaf spots follow. In several years of inoculation work on young plants in the bed, on green house plants, and on plants in the field, we have failed to find any but physiologically very young or very old leaves, which could not be water-soaked (under proper light conditions), and infected by pouring a suspension of bacteria over the water-soaked surface. In all instances, spots typical of the organisms used (*Bacterium angulatum* or *Bact. tabacum*) developed in the usual time. It may be stated, therefore, that the presence or absence of the diseases in the plant bed, if weather conditions have been favorable, depends not on the nutrition of the plant, as Reid *et al.* claim, but on the presence or absence of the organisms on the roots of plants. Considering the persistence of the pathogenic organisms in the soil, which has been demonstrated in the present studies, it seems unnecessary here to assume that the pathogenic organisms are being derived, in a relatively short period of time, from the nonpathogenic *Pseudomonas fluorescens*. If this is ever proved to occur, then the rate of change and its frequency must be studied and demonstrated before it can be concluded that *P. fluorescens* is a danger in old tobacco-growing areas.

The present study has demonstrated that the pathogenic bacteria form colonies, on the roots of plants, which can be found by microscopic examination and that, upon isolation of the bit of root, typical leaf spots can be produced on tobacco leaves, if it is used as inoculum. The fact that the bacterial leaf-spot diseases of tobacco are found in every country where tobacco is grown suggests that these pathogenic bacteria probably are rather generally distributed over the world. It would not be surprising, therefore, if the very similar colonies photographed by Linford[8] on the roots of maize and

[8] Linford, M. B. Methods of observing soil flora and fauna associated with roots. Soil Science 53: 93–103. 1942.

lettuce were similar to or identical with *Bacterium tabacum* and *Bact. angulatum* or were a pathogen capable of causing a leaf disease on other species of plants than tobacco.

SUMMARY

Colonies of *Bacterium tabacum* and *Bact. angulatum* are found in nature on the roots of pasture and crop plants and can apparently maintain themselves indefinitely in this way. Soil collections, presumably containing roots bearing colonies of the pathogenic organisms, frequently cause wildfire or angular leaf spot in the usual time after inoculations are made, thus demonstrating that the organisms are present in the pathogenic form in the soil and apparently persist in this form. Colonies of bacteria can be found on rootlets of infected tobacco plants from the bed or field, which, when used as inoculum, produce the disease present on the original plant. There appears to be no reason for assuming that outbreaks of either angular leaf spot or wildfire arise other than from colonies of the respective pathogenic organisms already present in the soil before outbreaks occur.

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THE USE OF ETHYL MERCURY PHOSPHATE FOR TREATING TOMATO SEED IN NEW JERSEY[1]

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INTRODUCTION

For many years various seed treatments have been used to destroy organisms carried on or within the seed. Chemical compounds, such as copper sulphate, mercuric chloride, and Semesan, have been used for surface disinfection of tomato seeds with a considerable degree of success (2). Hot-water treatment, since it is effective against internal infection, also has been and still is recommended by some workers (1, 3).

Most of these treatments have one important disadvantage in that they do not protect the seed against recontamination after it has been treated. The introduction of organic mercury compounds, particularly New Improved Ceresan (5% ethyl mercury phosphate), has supplied the seedsman with a treatment which effectively disinfects the surface of the seed (6) and leaves a coating of the chemical, which to some extent protects the very young seedling against pre-emergence damping-off caused by soil organisms. As a result of the effective surface disinfection obtained with ethyl mercury phosphate it has come into rather general use. This has led to the necessity of working out efficient and economical methods of treatment particularly for those seedsmen and canners who handle large quantities of seed. The experiments here described deal chiefly with studies of such treatments.

A large quantity of certified tomato seed is produced each year in New Jersey, and a considerable portion of it is treated with some disinfectant to control surface-borne organisms. This is done chiefly to comply with the regulations of various foreign countries to which the seed is shipped and to meet the requirements for the production of State-certified tomato plants grown in Georgia for shipment to the North.

Bacterial spot (*Phytomonas vesicatoria* (Doidge) Bergey *et al.*) is not common on tomatoes in New Jersey. Bacterial canker (*Phytomonas michiganense* (E.F.S.) Bergey *et al.*) is found occasionally, but it, ordinarily, is not a factor to be reckoned with in treatment of tomato seed, since all certified seed produced is taken from fields certified for freedom from that disease. Septoria leaf blight (*Septoria lycopersici* Speg.) occurs to some extent in some years, but is not of major importance in most seasons. Bacterial wilt (*Phytomonas solanacearum* (E.F.S.) Bergey *et al.*) and anthracnose (*Colletotrichum phomoides* (Sacc.) Chester) occur each year, but do not

[1] Cooperative investigation between the U. S. Department of Agriculture, the agricultural experiment stations of New Jersey, Purdue University (Ind.), Georgia, the Georgia Coastal Plain Experiment Station, and the Georgia Department of Entomology.

[2] Formerly, Agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

appear to be seed-borne. Early blight (*Alternaria solani* (E. and M.) J. and G.) occurs commonly every season, and, from the point of view of both the plant grower and seedsman, is by far the most important disease in tomatoes. Massie (4) and Samson (5) have reported cases of infection of tomato seed by *A. solani,* but this disease rarely is seen on the fruits in New Jersey, and in plating perhaps 75,000 seeds surface-sterilized by various means, neither *Alternaria solani* nor any other tomato pathogen was ever encountered. It, therefore, appears that under New Jersey conditions the efficient surface disinfection of tomato seed generally will insure a high degree of protection against seed transmission of pathogenic bacteria and fungi.

MATERIALS AND METHODS

Throughout the experiments reported in this paper all tomato seed were treated at the rate of 1 lb. of dry seed or 1 qt. of wet seed (recovered from pomace left after fruit is processed for the manufacture of tomato juice) per gallon of treating solution. Except where temperatures are specifically stated, all solutions were made with tap water at a temperature of approximately 60° to 65° F. The standard 1–24,000 solution of ethyl mercury phosphate was prepared by dissolving 1 g. of New Improved Ceresan in 1200 cc. of water. Where dusts were applied the amounts recommended by the manufacturer were used. After thorough shaking of seed and dust, all excess was screened off from the seed.

All seeds were plated on potato-dextrose agar (1.8 per cent agar) and were held seven days before final counts of contaminated seeds were made. All of the germination data recorded are for germination in soil, since quantities of chemicals, too small to interfere with germination in soils, often cause marked retardation of germination on blotters. In each instance the location and type of germination medium have been recorded.

Germination of Tomato Seed Treated with Ethyl Mercury Phosphate as Compared with Seed Treated with Other Standard Seed Treatments

In studying seed treatment with ethyl mercury phosphate, preliminary tests were made to compare its effect on germination with other standard seed treatments. These tests included the following treatments; corrosive sublimate 1–3000 for 10 minutes, followed by a 30-minute wash in running water before drying; ethyl mercury phosphate 1–24,000 for 5 minutes, then dried immediately; ethyl mercury phosphate 1–24,000 for 5 minutes, dried immediately, then treated with either red copper oxide dust or with Vasco-4 dust; ethyl mercury tartrate 1–16,000 for 5 minutes, then dried immediately; mercurous oxide on bentonite 1–32,000 for 5 minutes, then dried immediately; metallic mercury on lampblack (dust); and hot water (55° C. for 10 minutes). All seed was stirred vigorously, while submerged in the treating solution, to be sure that all aggregates of seeds were broken up and all seed surfaces brought in contact with the treating solution. These trials

were conducted in 1938 and 1941, and 300 to 500 seeds per treatment were planted. New Jersey certified seed of a strain of Marglobe tomato was used. The seeds were planted in the greenhouse in a Sassafras sandy loam soil. The results from all trials showed that there was no significant difference in germination between any of the treatments and the untreated checks. In 1941 samples of the seed used in the experiments were also tested to determine the degree of surface disinfection resulting from the various treatments. With the exception of the seed treated with hot water, where 11 per cent of contamination occurred, all of the treated samples showed 99 to 100 per cent of the seed free from surface contaminants. The results with ethyl mercury phosphate are in agreement with those of Samson (6) who reports that a 1–32,000 solution of 5 per cent ethyl mercury phosphate gave complete surface disinfection of tomato seed and did not reduce the germination below that obtained from seed treated with 1–3,000 solution of mercuric chloride.

The results of all the comparative tests mentioned above have confirmed the preliminary data, which indicate that ethyl mercury phosphate is an effective surface disinfectant for tomato seed and when used in the proper concentrations is not likely to cause any serious reduction in germination.

Use of Dyes for Seed Treatment

Since certain seedsmen add dyes to the treating solution to distinguish treated from untreated seed, and since many dyestuffs have fungicidal and germicidal properties, seeds treated with various concentrations of safranine A, methyl violet 2B, brilliant-green B, and chrysoidine 3R plus safranine A were plated on potato-dextrose agar to determine whether or not the dyes alone might be of value as seed disinfectants. Of these dyes only brilliant-green B appeared to have sufficient germicidal strength to give adequate disinfection; and, in order to obtain satisfactory disinfection with it, a solution of such great concentration was necessary that the cost was prohibitive.

Effect of Repeated Use of Solutions of Ethyl Mercury Phosphate in Seed Treatment

Where seed is treated in large quantities there has been a question as to whether the solution would retain its effectiveness if used for the treatment of more than one lot of seed. To test this, successive lots of seed were treated in the same solution and samples of the respective seed lots were then plated to determine the amount of surface contamination still present. The seed was treated for 5 minutes at the rate of 1 lb. of dry seed to 1 gal. of solution, and then removed and spread out to dry. The results of these tests (Table 1) indicate that only a single lot of seed should be treated and the solution should then be discarded.

Effect of Concentration of the Treating Solution on Disinfection and Germination of Seed

Three series of tests were conducted to determine the concentration of ethyl mercury phosphate solution necessary for adequate surface disinfec-

TABLE 1.—*Results of treating successive lots of tomato seed in the same solution of ethyl mercury phosphate, 1–24,000*

Seed lot	Per cent of seed free from contamination	
	First test[a]	Second test[b]
1st	97.5	100
2nd	91.0	97
3rd	71.1	91
4th	.....	47

[a] Treatments made by a commercial seedsman.
[b] Treatments made in the laboratory at 60° F.

tion with a minimum reduction in germination. From the results of the first test (Table 2) it was evident that concentrations greater than 1–20,000 would seriously interfere with germination of the seed. When the greater

TABLE 2.—*Germination of seed treated for 10 minutes with various concentrations of ethyl mercury phosphate at the rate of one pound of dry seed to one gallon of solution. Seed planted in flats of sassafras sandy loam in the greenhouse*

Concentration	Germination	Number days until rows of plants were clearly visible
1st planting[a]	*Per cent*	
1–12,000	61	17
1–16,000	62	15
1–20,000	82	13
1–24,000	84	13
Untreated check	74	12
2nd planting[b]		
1–12,000	63	15
1–16,000	68	13
1–20,000	87	11
1–24,000	91	10
Untreated check	88	9

[a] April 1941.
[b] June 1941.

concentrations were applied the percentage of germination was reduced by as much as 20 to 25 per cent as compared with nontreated seed, and germination was delayed from 3 to 6 days.

TABLE 3.—*Results of treating tomato seed for 10 minutes with various concentrations of ethyl mercury phosphate at the rate of one pound of dry seed to each gallon of solution*

Concentration of solution	Per cent free from contamination	
	1st series	2nd series
1–16,000	.....	100
1–20,000	100	.....
1–24,000	100	100
1–28,000	99	.....
1–32,000	99	100
1–40,000	.....	100
1–48,000	.....	98
Untreated check	0	0

The second and third tests (Table 3) showed that it was not necessary to have solutions of concentrations greater than 1–24,000 in order to secure complete surface sterilization. Since a 1–24,000 solution gave adequate surface sterilization without interfering with germination other than to delay it by approximately one day, this concentration was used in all subsequent experiments.

Length of Exposure to Treatment

Although a 5-minute treatment in the laboratory gave perfect control of surface contamination, in each lot of commercially treated seed a small percentage was contaminated. It seems probable that this is because the centrifugal "wringers" used in commercial seed treating throw off the solution before sufficient mercury has been adsorbed. Two methods of overcoming this difficulty have been found to be entirely satisfactory. Either the exposure can be increased to 10 minutes or the sacks of seed can be left to drain for 20 to 30 minutes before they are placed in the "wringers." When a 1–24,000 solution is used, neither of these practices causes injury to the seed.

Temperature of the Treating Solution

In treating commercial seed in New Jersey, tap water or water drawn from streams is used to make up the solutions and the temperature varies according to the weather at the time of treating. To determine the influence of the temperature of the treating solution on the effectiveness of the treatment, seeds were treated in 1–24,000 ethyl mercury phosphate at temperatures of 43°, 60°, and 80° F. for 5 minutes and 10 minutes, both with and without a draining period before they were placed in the centrifuge. The results of 3 experiments (Table 4) indicated that the temperature of the solution is not of primary importance, since the seeds treated in the solution at 43° F. were as free from surface contamination as those treated in the solution at 80° F. and the germination of the seed treated at the higher temperatures was not significantly lower than that of seed treated at 43° F.

TREATMENT OF FRESHLY EXTRACTED *VS.* DRIED SEED

During wet weather the cost of drying large quantities of seed is very great, and some seedsmen would like to eliminate, if possible, one drying operation. Therefore, experiments were conducted to determine the comparative effectiveness of the treatment of freshly extracted, moist seed, and of seed that had been dried before treatment. In 1938 tomato seed was extracted by fermenting for 36 hours and then washing in water. The seed was divided into 2 lots, one treated immediately, the other dried for 7 days before treating. As shown in table 5, treating immediately after extraction did not reduce the germination of the seed to any appreciable extent, although treating with ethyl mercury phosphate has been shown to retard germination one or two days. Platings showed that all of the lots of treated seed were free from surface contamination.

TABLE 4.—*Influence of temperature of the treating solution upon the effectiveness of seed treatment with 1–24,000 ethyl mercury phosphate*

Length of exposure to treating solution	Drained 30 minutes before centrifuging	Temperature of treating solution	Free from contamination[a]	Germination after 14 days[b]
		Degrees F.	*Per cent*	*Per cent*
1st Experiment				
5 min.	No	43	100	85
5 "	"	60	100	84
5 "	"	80	100	81
5 "	Yes	43	100	82
5 "	"	60	100	87
5 "	"	80	100	85
10 "	No	43	100	80
10 "	"	60	100	84
10 "	"	80	100	81
10 "	Yes	43	100	77
10 "	"	60	99	85
10 "	"	80	100	87
Untreated check	.....	.....	0	79
2nd Experiment				
5 min.	No	45	100	84
5 "	"	60	100	82
5 "	"	80	100	82
10 "	"	45	98	80
10 "	"	60	100	85
10 "	"	80	99	79
Untreated check	.....	.....	0	70
3rd Experiment				
5 min.	No	50	100	84
5 "	"	70	99+	83
5 "	Yes	50	99+	81
5 "	"	70	100	85
10 "	No	50	100	84
10 "	"	70	100	84
Untreated check	.....	.....	0	81

[a] 300 seeds from each lot plated.
[b] 500 seeds from each lot planted in flats of soil in the greenhouse.

TABLE 5.—*Treatment of tomato seed with ethyl mercury phosphate, with ethyl mercury tartrate, or with $HgCl_2$ immediately after extraction, and after thorough drying*

Time of treatment	Treatment	Germination after 14 days[a]	Free from contamination[b]
		Per cent	*Per cent*
First experiment			
Immediately after extraction	Ethyl mercury phosphate 1–24,000	91.1	100
" " "	$HgCl_2$ 1–3,000	92.1	100
After drying	Ethyl mercury phosphate 1–24,000	92.5	100
" "	$HgCl_2$ 1–3,000	93.0	100
Check	None	91.3	5
Second experiment			
Immediately after extraction	Ethyl mercury phosphate 1–24,000	98.1	100
" " "	Ethyl mercury tartrate 1–15,000	97.1	100
" " "	$HgCl_2$ 1–2,500	96.8	99
Check	None	96.4	11

[a] 750 seeds from each lot planted in flats of soil in the greenhouse.
[b] 250 seeds from each lot plated.

A second lot of seed, also extracted by fermentation, was treated immediately after extraction. Platings showed that all lots of the treated seed were free from surface contamination. Table 5 shows that treatment immediately after extraction did not reduce the percentage of germination of the seed. In this experiment ethyl mercury tartrate was tested for comparison with ethyl mercury phosphate and gave perfect control of surface contamination without interfering with germination of the seed. However, since it did not appear to have any advantage over the commercially-available ethyl mercury phosphate it was not tested further.

Effect on Germination of Type of Container Used for Storing Seed Treated with Ethyl Mercury Phosphate

A third lot of seed, extracted by fermentation, was treated with a 1–24,000 solution of ethyl mercury phosphate or with a 1–3,000 solution of $HgCl_2$, part immediately after extraction and part after being thoroughly dried and stored for two weeks. Each lot was then divided into 3 parts, one of which was stored in ordinary paper bags, one in tight cardboard containers, and one in sealed jars. Plantings of 500 seeds each, made at intervals over a period of 2 years showed (Table 6) that the treatments, whether made immediately after extraction or after drying, do not seriously affect the germination of the seed. It also appears that seed treated in either the freshly extracted or the dried state can be stored in air-tight containers for periods up to 2 years without seriously affecting its germination. After 2 years the germination of all seed lots was considerably lower than at the beginning of the experiment. This reduction in germination was much more marked in the seed stored in paper bags than in either of the other types of containers. The laboratory in which the seeds were stored was perhaps warmer and more humid than most seed storage rooms, and it is probable that the reduction in germination was greater than would occur in commercial seed storage.

Since the above tests with freshly extracted seed were all made in the laboratory and involved only small lots of seed, it seemed advisable to repeat part of the experiment using larger amounts of seed and making the treatments under conditions comparable with treatments made by commercial seedsmen. A quantity of pomace was, therefore, obtained, a part of which was extracted immediately and a part put aside to ferment for 24 hours before being washed out. Each of these lots was again divided into 2 parts, one for treating immediately and the other for treatment after having been thoroughly dried. Two identical experiments were conducted on this phase of the problem.

Since the treatments were made at a cannery where highly sensitive scales were not available, concentrations slightly different from those ordinarily recommended had to be used (ethyl mercury phosphate at the rate of 1–20,480 instead of 1–24,000, and $HgCl_2$ at the rate of 1–3,072 instead of the usual 1–3,000).

The data in table 7 show that, with the exception of 2 or 3 unexplainable instances in one experiment neither of these chemicals seriously interfered with the germination of the seed, regardless of whether they were applied immediately after extraction or after the seed had been thoroughly dried. This was also true with regard to the method of extraction, since no appreciable differences were observed in the germination of the seed extracted by the two methods.

TABLE 6.—*Germination of tomato seed treated immediately after extraction and after thorough drying, when stored in various types of containers*

Type of container	Time of treatment	Treatment	Germination after storage for		
			2 weeks	1 year	2 years
			Per cent	*Per cent*	*Per cent*
Paper bags	Immediately after extraction	Ethyl mercury phosphate 1–24,000 for 5 minutes	89.3	86.3	43.4
Paper bags	Immediately after extraction	$HgCl_2$ 1–3,000 for 5 minutes	84.0	86.3	50.0
Paper bags	After drying	Ethyl mercury phosphate 1–24,000 for 5 minutes	86.2	83.0	53.4
Paper bags	After drying	$HgCl_2$ 1–3,000 for 5 minutes	88.3	87.7	42.6
Paper bags	Check	None	83.7	98.7	30.2
Cardboard cartons	Immediately after extraction	Ethyl mercury phosphate 1–24,000 for 5 minutes	87.1	87.0	70.8
Cardboard cartons	Immediately after extraction	$HgCl_2$ 1–3,000 for 5 minutes	86.3	86.7	72.0
Cardboard cartons	After drying	Ethyl mercury phosphate 1–24,000 for 5 minutes	87.6	88.0	82.4
Cardboard cartons	After drying	$HgCl_2$ 1–3,000 for 5 minutes	88.8	89.3	70.0
Cardboard cartons	Check	None	86.1	90.0	55.8
Sealed jars	Immediately after extraction	Ethyl mercury phosphate 1–24,000 for 5 minutes	87.6	84.7	77.2
Sealed jars	Immediately after extraction	$HgCl_2$ 1–3,000 for 5 minutes	87.2	86.7	42.2
Sealed jars	After drying	Ethyl mercury phosphate 1–24,000 for 5 minutes	91.2	83.0	50.8
Sealed jars	After drying	$HgCl_2$ 1–3,000 for 5 minutes	88.1	82.0	73.6
Sealed jars	Check	None	98.9	84.7	61.2

In 1941, numerous platings also were made of seed treated for 10 minutes in a 1–24,000 solution of ethyl mercury phosphate by a commercial seedsman. Part of the seed was treated at the time of extraction and part several weeks later. All lots tested were free from surface contamination.

These experiments indicate that treatment of tomato seed with either 1–3,000 $HgCl_2$ or 1–24,000 ethyl mercury phosphate can be carried on either at the time of extraction or at a later date, regardless of the method of extraction, with equally successful control of surface contamination and

with equal assurance that the treatment will not seriously interfere with the germination of the seed.

TABLE 7.—*Germination of tomato seed under conditions comparable with treatment by commercial seedsmen*[a]

Method of extraction and treatment applied	Germination			
	First experiment		Second experiment	
	Greenhouse, 18 days	Field, 19 days	Greenhouse, 18 days	Field, 19 days
	Per cent	*Per cent*	*Per cent*	*Per cent*
Not fermented				
Treated immediately; ethyl mercury phosphate 1–20,480 for 5 minutes	68.6	49.6	99.2	64.0
Treated immediately; $HgCl_2$ 1–3,072 for 5 minutes	86.0	55.0	93.4	71.3
Dried, treated later; ethyl mercury phosphate 1–20,480 for 5 minutes	90.2	62.8	92.0	82.0
Dried, treated later; $HgCl_2$ 1–3,072 for 5 minutes	85.4	58.8	94.8	64.4
Check—not treated	85.8	65.6	80.2	55.3
Extracted by fermentation				
Treated immediately; ethyl mercury phosphate 1–20,480 for 5 minutes	74.2	68.8	87.2	78.6
Treated immediately; $HgCl_2$ 1–3,072 for 5 minutes	88.4	58.0	90.8	76.8
Dried, treated later; ethyl mercury phosphate 1–20,480 for 5 minutes	89.4	60.6	85.4	77.0
Dried, treated later; $HgCl_2$ 1–3,072 for 5 minutes	90.0	45.2	92.4	69.8
Check—not treated	86.2	64.4	79.0	72.4
Standard error	9.129±	18.280±	8.811±	12.229±

[a] All treated lots were free from surface contamination.

SUMMARY

Ethyl mercury phosphate has proved as effective as mercuric chloride as a means of surface disinfection for tomato seed and it possesses the added advantage that a residue of the organic mercury compound may be left safely on the seed coat. This tends to prevent recontamination of the seed and affords a certain degree of protection against seedling damping-off.

Concentrations of ethyl mercury phosphate greater than 1–20,000 cause a reduction in the percentage of germination as well as a slowing up of the rate of germination. Since this concentration controls surface contamination as well as stronger concentrations without seriously interfering with the germination of the seed, it can be recommended for general use.

Solutions of ethyl mercury phosphate become less effective with repeated use and should be discarded after one treatment.

A 5-minute treatment has given perfect results in laboratory tests but when commercially treated seed is centrifuged immediately after treatment a period of 10 minutes will allow greater adsorption of the mercury compound and give more perfect surface disinfection. If the sacks of seed are

allowed to drain for 20 to 30 minutes before centrifuging, a 5-minute treatment will probably be effective.

The treating solution is equally effective at temperatures from 43° to 80° F. Tap water or water drawn from streams during the growing season is satisfactory for making up the solution.

Tomato seed can be treated in solutions of the above strength either at the time of extraction or after having been thoroughly dried, and may be stored from one season to the next in any type of container that is satisfactory for nontreated seed.

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EFFICACY OF FUNGICIDAL TRANSPLANTING LIQUIDS FOR CONTROL OF CLUBROOT OF CABBAGE[1]

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The clubroot organism (*Plasmodiophora brassicae* Wor.) is particularly difficult to control in acid soils. Although raising the pH of the soil solution to neutral or slightly alkaline by application of lime is sometimes effective, it is not always possible to maintain the reaction at such points with sufficient constancy to prevent infection (10, 24). Moreover, on acid muck the buffer action of the soil is sometimes such as to preclude any successful control by this method (27).

A number of workers have tried to control the disease in seed beds by applying fungicides broadcast along the row, but results have not been consistently successful (1, 2, 3, 4, 6, 7, 8, 9, 26), and such methods are, of course, applicable only to plant culture in cold frames or greenhouses. In 1927 Preston (13) offered a new approach when he reported reduction of the severity of the disease by adding mercuric chloride to the transplanting liquid in the dibble hole when plants of cabbage (*Brassica oleracea* var. *capitata* L.) were set in infested soil in the field. This method had been tried with some success by Halsted (3) and by Kindshoven (8), but neither of these investigators seems to have found it worthy of general recommendation. Preston (14, 15, 16, 17, 18) presented further results with other compounds, but mercuric chloride was in each case the most effective. Smieton (21) tried with some success pentachloronitrobenzene and trichloronitrobenzene mixed with soil in seed-boxes and in the dibble hole, but mercuric chloride was the most effective treatment with out-of-door plants. In Russia, Rochlin (19) reported that volatile mustard oil was toxic to the clubroot organism and that a crude extract of leaves and stems of black mustard (*Brassica nigra* L.) when added to the soil around young plants reduced the amount of infection that occurred. Preston (17) did not secure any beneficial results by watering mustard plants growing in infected soil with 0.05 and 0.0125 per cent allyl isothiocyanate, the sulphur oil most prevalent in mustard leaves. Immersing cabbage seedlings for 3 to 46 hours in the same solutions before setting on infested soil also failed to reduce clubroot infection. He also tried the incorporation into the soil in the dibble hole of 3 compounds: (1) calomel (HgCl); (2) an amalgam in the form of a fine powder

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containing 12 per cent metallic mercury and 6 per cent zinc and 82 per cent calcium carbonate; (3) a powder form of chloronitrobenzene known under the proprietary name of brassican. All of these reduced infection substantially, but none was superior to mercuric chloride in effectiveness. Indine blue, auramine, malachite green, and magnesium sulphate used as dusts in the soil of the dibble hole were of no value. The use of mercuric chloride in the transplanting water, with marked success, has been reported by others in England (11, 20, 22, 23).

The writers conducted the present study in 1938 to 1941. The purpose was to test the efficacy of mercuric chloride and mustard oil as fungicides in the transplanting liquid on mineral and muck soils and to explore the possibility of other compounds that might be used. The work was carried on in the greenhouse at Madison, Wisconsin, and in the field in commercial cabbage areas in Racine and Kenosha Counties, Wisconsin. A preliminary report has already been published (25). Experiments in New York have been reported by Palmer (12).

METHODS AND MATERIALS

Two types of soil—mineral and muck—were used, since cabbage culture is common to both. A field of Clyde silt loam in Kenosha County, known to be severely infested, was used as representing the first type, while most of the trials on muck were made in a tract near Wind Lake, Racine County.

In the early experiments, which included numerous chemicals and dosages, single-row plots of 20 to 30 plants each were employed. The usual transplanting hole was made, the specified amount of liquid applied, and a plant from a healthy seed bed set and covered. After the normal time for recovery and renewed growth, note of injury to the plant was taken from the appearance of the aboveground portion of the plant. Four grades of plant injury were arbitrarily established extending from 0 for no injury to 3 for severe injury. At harvest all plants in the plot were pulled, and each placed in one of 5 classes of disease based on the extent of severity, from no infection to very severe clubbing. On the basis of such grades, which were given equal weight, a disease index for each plot was calculated. The index might vary from 0 for all clean plants to 100 for all severely clubbed plants. Heads were sometimes removed and weighed for the determination of yield.

When large trials on a commercial scale were conducted, the results usually were secured by pulling plants at random in each treatment and determining the disease index. When yields were estimated, blocks of 25 heads were cut at intervals and weights secured.

Chemicals used were all secured from reliable manufacturers.

Preliminary tests with many of the chemicals were made in the greenhouse in order to determine the relative range of toxicity to the host and to the pathogen. Infested muck soil from the Wind Lake area was used in 6-inch clay pots. Six to 10 cabbage plants in the third- to fourth-leaf

stage were used as test plants in each pot. Five ml. of a solution or uniform suspension of the chemical were placed in the small hole made for each plant. Whereas data secured from these tests could not be applied directly to field performance, they did give opportunity to test in a preliminary way the relative value of an unknown in comparison with a substance on which a field reading had been made.

EXPERIMENTAL RESULTS

Comparison of Allyl Isothiocyanate and Mercuric Chloride

In 1938 allyl isothiocyanate and mercuric chloride were compared in field trials on naturally infested Clyde silt loam soil in Kenosha County, Wisconsin. Cabbage plants were set on June 18 with 425 ml. of liquid per plant in single-row plots of 20 plants with 14 replicates of each treatment. Final results, taken on September 29, are presented in table 1. It will be seen

TABLE 1.—*Comparison of clubroot development on cabbage when transplanted with liquid containing allyl isothiocyanate, in various amounts, or mercuric chloride. (Average of 14 replicates per treatment)*

Agent	Concentration	Disease development	Plants headed	Average wt. per head	Yield per acre
	p.p.m.	*Index*	*Per cent*	*Lb.*	*Tons*
None	.....	100.0	0.0	0.0	0.0
Allyl isothiocyanate	50	97.9	7.0	3.2	0.76
	125	92.9	9.8	2.3	0.80
	250	93.4	5.6	1.8	0.35
	500	91.4	17.3	2.1	1.35
	1000	87.3	17.4	2.3	1.45
Mercuric chloride	667	27.2	74.4	4.2	12.35

that mercuric chloride at 667 p.p.m. (1–1500) was very effective in reducing disease development. A large percentage of the plants produced good-sized heads, and a yield of 12.35 tons per acre was harvested. Although in greenhouse trials, reported elsewhere (5), concentrations from 10 to 350 p.p.m. of allyl isothiacyanate in inoculum of spore suspensions prevented infection, very little effective control was secured in the field when concentrations up to 1,000 p.p.m. were used in the transplanting water. Moreover, at the highest concentrations there was considerable injury to the plants.

The appearance of a typical plot treated with mercuric chloride is shown in figure 1. When the plants were pulled and examined, it was found that the chief benefit of the treatment was in the protection of the young rootlets that were produced in abundance from the pericycle of the tap root when the plant was set into moist soil. Ordinarily, the high soil moisture thus provided favored not only root development by the host but spore germination by the pathogen. Thus, heavy club formation occurred immediately around the tap root of the control plant and the feeding capacity of the root system was rapidly impaired. When an effective fungicide was provided in the

transplanting liquid, infection of the plant at this vital locus was largely prevented. Although clubs formed later at various points on the root system, the detrimental effect on plant growth was much less pronounced. In figure 2 are shown a plant from an untreated row and one from a mercuric chloride-treated row, collected at the time of harvest. Many of the numerous clubs on the former had decayed, and the functional feeding roots were few. In the latter a functional root system still remained regardless of clubs formed at various scattered points.

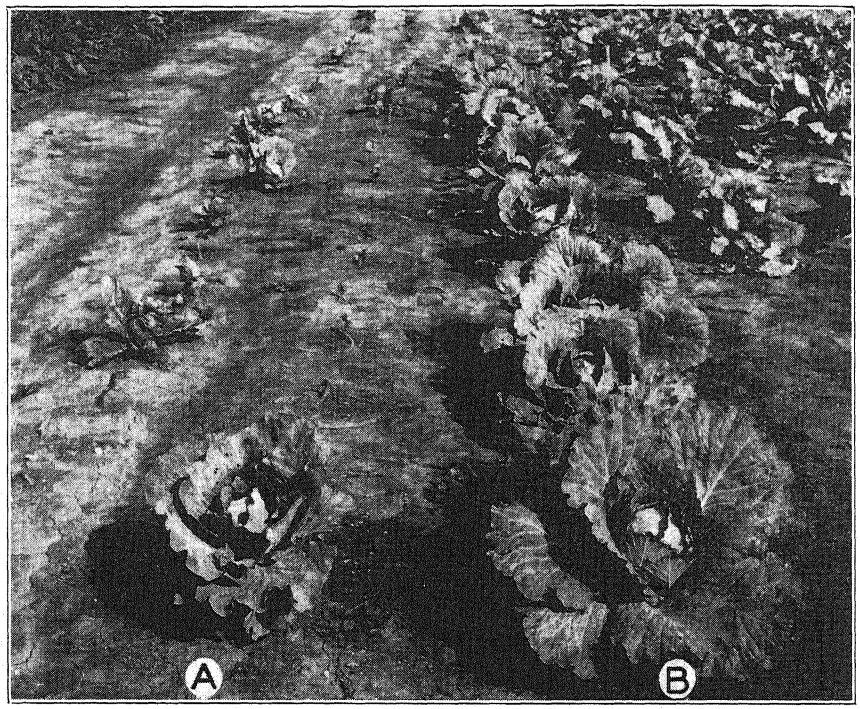


FIG. 1. Effect of use of mercuric chloride on control of clubroot in heavily infested soil. A. Plants, at the end of the season, which had been transplanted with water (425 ml. per plant). B. Plants transplanted with mercuric chloride (1–1500 solution, 425 ml. per plant).

Exploratory Trials With Various Fungicides

In 1939 trials were made with a considerable number of other fungicides added to the transplanting liquid. Most of these were tested in the greenhouse during the winter of 1938–1939. Since the volume of 425 ml. per plant used in the 1938 trials was much higher than that practicable in commercial culture, the dosage was reduced to 125 ml., which was close to the average rate in general use. Various concentrations were used, the range being designed to include at the upper limit a point at which host injury was marked or the cost prohibitive. The trials were made with 26-plant plots on heavily infested muck soil with a pH of 5.7 to 6.1. One plot per

treatment was employed and nontreated rows were included at frequent intervals. The uniformity of infestation is shown by the disease indices of the nontreated plots given in table 2. The inorganic and organic salts of mercury used, the concentrations used, the degree of plant injury, and the disease indices are given in table 2. Results with other organic and inorganic salts are given in table 3. It will be seen that $HgCl_2$, HgCl, HgO, and mercury pentachlorophenolate were superior to other mercury salts,

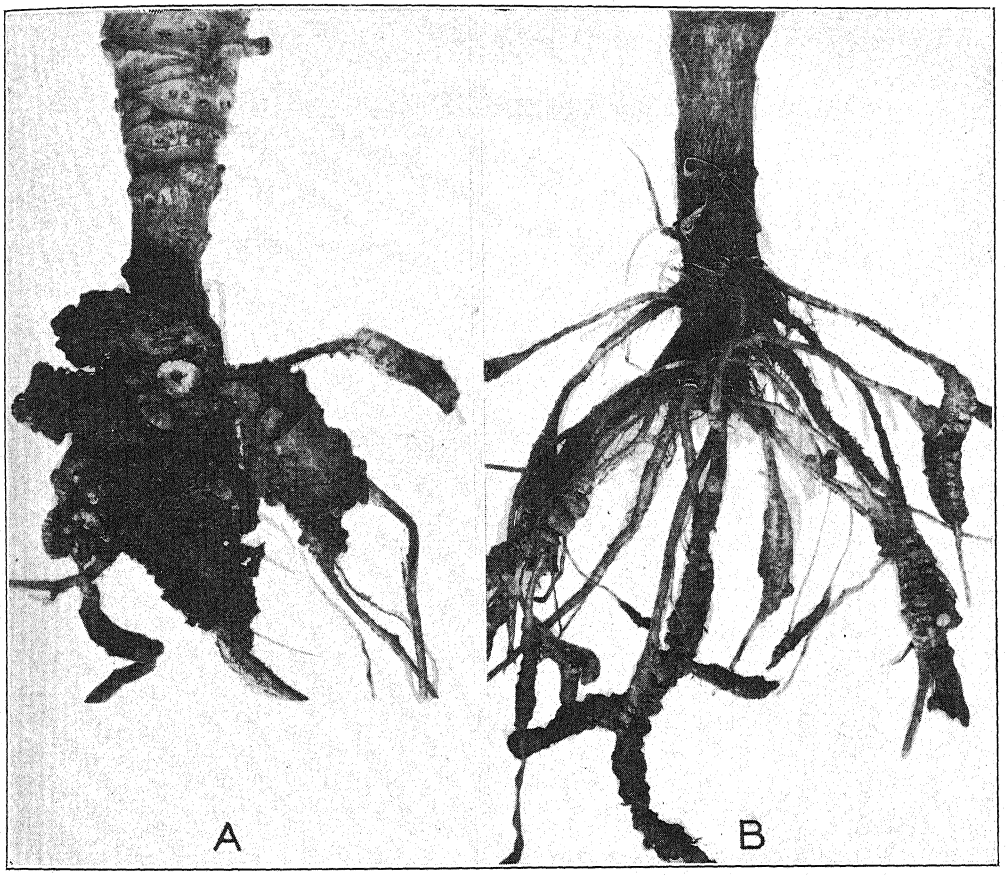


FIG. 2. Comparison of the root systems of untreated and mercuric chloride-treated plants at the end of the season. A. Surviving plant from an untreated row (see figure 1, A) with a few decayed clubs remaining and no functional feeding roots. B. Plant from a treated row. Note that no clubs developed close to the tap root and a well-functioning root system with scattered clubs was formed.

and that little promise was afforded by any of the other materials used. The lack of control with chlorobenzenes was in contrast to more favorable results reported in England for these compounds by Smieton (21). $HgCl_2$ held an advantage over HgCl and HgO because of its solubility in water, while the other two, being low in solubility, had to be kept in suspension by an agitator during application. When HCl was used to facilitate dis-

solving $HgCl_2$, control was about the same, but the acidified solutions produced noticeably greater plant injury.

Relation of Volume and Concentration to Control

In another experiment in 1939 on the same tract of muck mentioned in the foregoing, a series of tests was made in which the amount of chemical

TABLE 2.—*Comparative value of compounds of mercury in reducing severity of clubroot of cabbage when used in the transplanting liquid applied at 125 ml. per plant*

Compound	Grams per liter	Plant injury	Disease index	Compound	Grams per liter	Plant injury	Disease index
$HgCl_2$	10.30	3	25.00	2% ethyl mercury chloride (2% Ceresan)	7.60	2	89.42
	5.17	3	17.31		3.80	0	64.71
	3.48	2	15.28		1.52	0	77.40
	3.10	2	23.86		0.60	0	72.92
	2.90	1	29.55	10% hydroxymercurinitrophenol and 2% hydroxymercurichlorophenol (Semesan Bel)	1.64	0	63.54
	1.74	1	29.29		0.82	0	78.80
	1.50	1	22.22		0.41	0	80.88
	0.87	1	31.59				
	0.35	0	41.62				
	0.14	0	54.17				
$HgCl_2$:HCl	1.74: 8.8	2	16.67				
	1.74: 4.4	3	25.00	Hg. 2, 4, 5-trichlorophenolate	0.60	0	35.00
	1.74: 2.4	2	22.22		0.30	0	46.90
	1.74: 1.2	1	26.14		0.15	0	80.77
	0.87: 4.4	1	43.06		0.08	0	89.42
	0.87: 2.4	0	27.38		0.04	0	84.26
	0.87: 1.2	1	30.97	Hg penta-[a] chlorophenolate	1.60	1	35.00
	0.35: 2.4	1	41.67		0.80	1	36.07
	0.35: 1.2	1	38.09		0.40	0	39.00
HgCl	3.08	0	25.00	Hg ortho-[a] phenylphenol	2.00	0	23.91
	1.54	0	23.91		1.00	0	43.79
	0.77	0	27.05		0.50	0	84.62
	0.38	0	35.79		0.25	0	82.69
	0.19	0	45.19		0.12	0	94.23
	0.10	0	55.21				
HgO	1.30	0	27.11	None	.....	0	91.67
	0.65	0	38.07		.....	0	94.23
	0.32	0	29.35		.....	0	93.27
	0.16	0	50.00		.....	0	98.08
					.....	0	94.23
HgS	1.24	0	94.23		.....	0	90.38
	0.62	0	96.64		.....	0	87.50
	0.31	0	89.42		.....	0	92.31
	0.16	0	95.19		.....	0	88.46
					.....	0	94.23
					.....	0	97.12

[a] Crude product obtained by fusing a mixture containing equivalent quantities of the phenol concerned and mercurous oxide. These mercury phenolates were not available from commercial sources.

and the volume of liquid per plant were varied with each of 3 materials—mercuric chloride, ethyl mercury chloride, and 2, 4, 5-trichlorophenol. Three replicate blocks were set up in each of which the treatments were arranged at random. The results are given in table 4. It will be seen that, in confirmation of the single-plot trials reported in tables 2 and 3, mercuric chloride was significantly superior to the other 2 chemicals. There

was no consistent, significant difference between the 3 volumes of liquid per plant when the amount of chemical remained constant. As the amount of mercuric chloride per plant increased, the disease index at each volume decreased. At 63 ml. per plant there was a significant decrease in index with each increase in total chemical applied. The differences were not significant at 125 ml. and 250 ml. per plant. The results indicated that the

TABLE 3.—*Comparative value of a number of non-mercury compounds in reducing severity of clubroot of cabbage when used in the transplanting liquid applied at 125 ml. per plant*

Compound	Grams per liter	Plant injury	Disease index	Compound	Grams per liter	Plant injury	Disease index
$CaCl_2$	100.0	3	62.50	Pentachlorophenol (Santophen 20)	0.80	1	85.58
	50.00	3	99.04		0.40	1	87.50
	25.00	1	89.42		0.20	0	76.45
					0.10	0	54.55
$Ca(OH)_2$	128.00	0	72.12		0.07	0	75.00
	64.00	1	86.54				
	32.00	0	61.90	Orthophenylphenol (Dowicide 1)	5.12	1	89.42
$ZnSO_4$	17.4	1	65.34		2.56	1	88.46
	8.7	0	71.56		1.28	1	79.17
	4.3	0	61.96		0.64	0	67.31
					0.32	0	62.50
$ZnCl_2$	11.3	1	77.88		0.16	0	69.23
	5.6	1	77.19				
	2.8	0	72.73	Chloro-2-phenyl-phenol (Dowicide 3)	1.28	1	91.35
					0.64	0	74.04
ZnO	36.4	0	59.02		0.32	0	61.00
	18.2	0	55.17		0.16	0	65.38
	9.1	0	64.89		0.08	0	68.27
$Al_2(SO_4)_3$	85.6	0	97.12	Na chloro-2-phenyl-phenate (Dowicide C)	5.12	1	79.81
	42.8	0	91.35		2.56	0	73.24
	21.4	0	85.00		1.28	0	62.68
	10.7	0	92.31		0.64	0	58.65
					0.32	0	53.85
2, 4, 5-trichloro-phenol (Dowicide 2)	1.28	2	95.20		0.16	0	68.27
	0.64	2	95.37				
	0.32	2	90.97	2, 4-dinitrochloro-benzene	0.40	2	95.19
	0.13	0	92.86		0.20	1	86.75
	0.05	0	81.73		0.10	0	77.88
Na 2, 4, 5-trichlorophenolate (Dowicide B)	2.56	2	98.08	Chlorobenzene (Brassican)	15.60	0	75.00
	1.28	2	97.12		7.80	0	69.57
	0.64	2	89.42		3.40	0	79.81
	0.32	0	84.41		1.70	0	77.88
	0.16	0	87.50		0.85	0	75.27
	0.08	0	78.85		0.42	0	71.15
2, 3, 4, 6-tetrachlorophenol (Dowicide 6)	0.40	1	92.31	None (see table 2)			
	0.20	0	82.76				
	0.10	0	85.58				
	0.05	0	82.69				

amount of chemical applied to each plant was the important criterion of successful control, and that no advantage resulted from increasing the volume of liquid above 63 ml. per plant. This fact, of course, is an important one in the application of this method on a large scale with mechanical transplanters, where the amount of liquid applied is usually 100 to 125 ml. per plant.

Commercial Application

Since the results already presented indicated that mercuric chloride was the most effective chemical, it was tried more extensively on a commercial

TABLE 4.—*The effect of various amounts of mercuric chloride, ethyl mercury chloride, and 2, 4, 5-trichlorophenol in the transplanting liquid upon the development of clubroot*

Chemical used	Amount of chemical applied per plant	Disease at end of season when the indicated amount of liquid per plant was used			Amount applied per acre	Cost per acre[a]
		63 ml.	125 ml.	250 ml.		
	Grams	*Index*	*Index*	*Index*	*Grams*	*Dollars*
None	.....	98.1	97.4	98.9	.....	.....
Mercuric chloride	0.218	20.0	30.7[b]	24.8[b]	1744	5.00
	0.088	38.6	31.7	32.9	704	2.00
	0.035	53.2	42.5	42.9	280	0.80
Ethyl mercury chloride (2% Ceresan)	0.475	88.5	64.7	58.9	3800	5.00
	0.190	74.8	77.4	89.1	1520	2.00
	0.075	83.3	72.9	85.7	600	0.80
2, 4, 5-trichlorophenol (Dowicide 2)	0.040	80.3	91.0	80.1	320	0.63
	0.015	90.1	92.9	81.4	120	0.25
	0.008	83.3	81.7	94.8	64	0.10

[a] Costs indicated are based on the rate of 8000 plants per acre and at the pre-war price of chemicals as follows: mercuric chloride, $2.87 per kilo; ethyl mercury chloride, $1.32 per kilo; 2, 4, 5-trichlorophenol, $1.98 per kilo.

[b] Difference in index required for significance (19: 1): between chemicals, 7.7; between amounts of chemical per plant or between amounts of liquid per plant within any one chemical, 13.3.

scale. In 1939 a trial on muck soil was conducted. Water was applied at the rate of 138 ml. per plant and there were approximately 8800 plants per acre. Mercuric chloride was added to make up the liquid at 1–1500 and 1–750 strengths. This amounted to 0.092 and 0.184 gram per plant. The results presented in table 5 show a marked reduction in disease index and increase of yield when the treated rows are compared with untreated. Although the index was lower and the yield higher with the 1–750 solution as compared with the 1–1500 treatment, the differences were not significant. In this instance, however, the increase in yield more than offset the increased cost of chemicals in the stronger treatment.

TABLE 5.—*The effect of mercuric chloride in the transplanting liquid upon clubroot development and yield in a commercial planting of cabbage on muck soil*

Concentration of mercuric chloride	Amount of liquid per acre	Amount of liquid per plant	Amount of mercuric chloride per plant	Amount of mercuric chloride per acre	Cost of mercuric chloride per acre[a]	Disease development[b]	Yield per acre[b]
	Gals.	*Ml.*	*Grams*	*Grams*	*Dollars*	*Index*	*Tons*
None	320	138	.....	.....	.....	85.3	7.82
1–1500	320	138	.092	810	2.32	36.7	12.51
1– 750	320	138	.184	1620	4.64	30.6	13.07
Difference required for significance (19: 1)	.....	.....	.....	.....	.....	11.3	2.02

[a] Cost is based on the prevailing wholesale price of mercuric chloride in 1939 of $2.87 per kilo.

[b] Disease development indices and yields are the means of 6 replicates.

Trials on a commercial scale were continued in 1940 and 1941. Four tests were made, 3 on muck and one on mineral soil. In one test mercurous chloride was compared with mercuric chloride. Disease indices, secured shortly before harvest, are given in table 6. Although mercurous chloride showed good promise in the exploratory tests (Table 2), it was definitely inferior to mercuric chloride in test 1 (Table 6). In all cases a decided reduction in disease index was secured with mercuric chloride. Concentrations of 1–750 and 1–1500 were, in general, the most satisfactory; usually, the differences between the two treatments, as in the commercial trial of 1940 (Table 5), probably were not significant. In tests 2, 3, and 4, HCl was used to facilitate dissolving the $HgCl_2$. No plant injury was noted on muck soil. In test 4 on mineral soil, however, there was some injury to plants in areas where the soil was comparatively dry at the time of transplanting.

TABLE 6.—*The effect of mercuric chloride and mercurous chloride in the transplanting liquid on clubroot development in commercial plantings*

Material added to transplanting liquid	Concentration of material in transplanting liquid[a]	Disease indices in tests indicated			
		Test 1, muck soil, 1940	Test 2, muck soil, 1941	Test 3, muck soil, 1941	Test 4, silt loam, 1941
None	.....	75.95	99.45	36.55	72.92
Mercuric chloride, $HgCl_2$	1– 375	.....	.....	.....	20.28
	1– 750	27.50	.....	16.70	24.10
	1–1500	48.08	58.51	21.95	15.91
	1–3000	51.42	.....	33.33	.....
Mercurous chloride, HgCl	1– 862	64.58	.....	.....	.....
	1–1725	63.46	.....	.....	.....
	1–3450	83.33	.....	.....	.....

[a] The liquid was applied at the approximate rate of 300 gal. per acre, and plants were set at about 8800 per acre.

DISCUSSION AND SUMMARY

The studies reported herein were conducted over a period of 4 seasons. They show conclusively that certain substantial benefits may be derived from the use of mercuric chloride in the transplanting liquid used for cabbage. The control accomplished is not complete but it is sufficiently effective to result often in increased yields many times the cost of treatment.

No encouraging results were secured with other inorganic or organic materials that were tested. For the time being it would appear that mercuric chloride alone is safe to recommend. It proved successful on both mineral and muck soils, and its effectiveness appears to be due to its fungicidal action in the soil zone around the tap root of the transplant. It is suggested that possibly mercury ionizes rapidly in the soil and permeates as a vapor for an appreciable distance. Mercury vapors arising from the soil have been shown to be toxic to certain potted plants in the greenhouse (28). This may account in part for the superiority of the mercury salts over nonmercury substances in this role.

The use of hydrochloric acid as a solvent is fraught with some danger of injury to the plants, especially on mineral soils. From the trials reported it would appear that the most effective treatments would be found within the range of 1–750 to 1–1500 applied at the rate of 60 to 125 ml. per plant.

There is nothing in the results reported to indicate that the control will be complete. Therefore, it should not be considered a substitute for such well-established practices as rotation, seed-bed sanitation, and, if feasible, maintenance of a neutral reaction by liming. As a supplement to these measures it should often be a worth-while procedure especially on mildly infested muck soils where the high buffer action often reduces or precludes any beneficial effect of liming in neutralizing the acidity of the soil solution.

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THE INFLUENCE OF GUTTATION FLUID ON PESTICIDES

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Since it was first discovered that Bordeaux mixture has fungicidal values, investigators have been perplexed by two problems involved in its use: (1) how does the insoluble copper in this mixture become soluble and therefore effective as a fungicide; and (2) how does this soluble copper at times enter and injure the leaf. The numerous publications of investigations within the past 60 years and the controversial nature of the reports are ample evidence of the complexity and importance of these problems.

There seems to be unanimity of opinion among investigators on several points: (1) copper, if effective as a fungicide, must be in a soluble or ionic form; (2) it does at times enter and injure the plant; (3) the copper in Bordeaux mixture is in an insoluble form; (4) some chemical action between the deposit and (a) the atmosphere, (b) meteoric water, (c) the fungus, or (d) the host plant or (e) other unknown agencies, to bring this insoluble copper into solution.

The object of this paper is to contribute certain new observations and experimental evidence that, when taken in consideration with the statements in the literature, may help to clarify these problems and may more fully explain the puzzling assortment of observations found in the literature concerning the physiological and chemical processes that are taking place between the pesticide and the host plant.

Since so many conflicting observations have been recorded concerning the capricious behavior of Bordeaux, only those references that may have a direct bearing on this investigation will be cited. Others (1, 2, 3, 6, 9, 10, 12, 13, 14, 17) have summarized quite adequately the observations and hypotheses previously advanced.

Barker and Gimingham (2) have grouped the various hypotheses concerning the solubility of copper in Bordeaux mixture, and its fungicidal value under these headings:

"1. The copper is brought into solution by atmospheric agencies—more especially by the action of the carbon dioxide of the air; *i.e.*, a purely chemical explanation.

"2. That the leaves, onto which the mixture is sprayed, exert a solvent action on the copper compounds, *i.e.*, an action of the host plant.

"3. That the fungus itself is responsible for the production of the soluble copper by which it is finally poisoned, *i.e.*, an action of the fungus."

McCallan (12, 13) and McCallan and Wilcoxon (14) attach little importance to CO_2 in the atmosphere or to uninjured leaves as having any solubilizing effect on copper in Bordeaux mixture. They do state, however, that meteoric water and injured or macerated leaves as well as fungous spores have the ability to release ionic copper in toxic concentrations from Bordeaux mixture.

TABLE 1.—*Chemical analysis of guttation fluid from plants*

Plant	Squash[a]	Tomato[b]	Tomato[c]	Squash[d]	Squash[e]	Cucumb's[f]	Cucumb's[g]	Squash[h]	Squash[i]	Cabbage[j]
Date	4–17–42	5–7–42	5–8–42	7–4–42	7–5–42	8–13–42	8–14–42	7–19–42	8–14–42	7–19–42
pH test	6.5	6.75	.....	7.30	7.10	6.00	6.48	6.30	7.30	6.08
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Chemical tests										
Nitrite nitrogen	.....	1.5	.....	.....	.....	1.0	1.0	.....	.....	.....
Nitrate nitrogen	250	1.0	1	1.0	10.0	3.0	1.0	5.0	13.0	4.0
Ammonia nitrogen	5	7.5	20	1.0	5.0	4.0	4.0	5.0	3.0	2.0
Phosphorus	75	2.0	5	2.0	1.0	8.0	8.0	1.0	1.5	4.0
Potassium	75	40.0	50	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Calcium	750	125.0	150	100.0	125.0	100.0	100.0	100.0	100.0	100.0
Magnesium	50	8.0	20	15.0	18.0	6.0	15.0	15.0	14.0	5.0
Aluminum	.....	0.3	.....	0.03	0.03	0.3	0.5	0.5	0.3	0.3
Manganese	.....	1.0	.....	T	T	T	.....	.....	T	1.0
Chlorine	.....	25.0	.....	.....	.....	50.0	25.0	25.0	25.0	35.0
Sulphate sulphur	.....	30.0	.....	.....	.....	25.0	25.0	25.0	25.0	25.0
Sodium	.....	T	.....	.....	.....	T	T	.....	.....	.....
Zinc	.....	.....	.....	5.0	5.0	.....	.....	.....	.....	.....
Copper	.....	.....	.....	.....	.....	.....	2.0	.....	.....	.....
Total solids	2500	600.0	.....	.....	.....	.....	.....	.....	.....	600.0
Organic	1100	275.0	.....	.....	.....	.....	.....	.....	.....	250.0

[a] Summer squash *Cucurbita pepo* growing in greenhouse 10 weeks old.
[b] Tomatoes growing in greenhouse 10 weeks old.
[c] Same as [b] except plants well fertilized with $(NH_4)_2SO_4$, treated with $(NH_4)_2SO_4$ on 5–6–42.
[d] Hubbard squash—Doolittle farm just before storm.
[e] Same as [d] except 1 day later.
[f] Cucumbers growing in greenhouse.
[g] Same as [f] except 1 day later.
[h] Summer squash—Mt. Carmel, Conn.
[i] Hubbard squash—same field as [d] and [e].
[j] Cabbage—Mt. Carmel, Conn.

It is with the second hypothesis that this paper is principally concerned. Wilson (18) pointed out that plants excrete fluid from uninjured leaves, which contains varying amounts of soluble materials. Table 1 gives the analysis of some of this fluid. This fluid or guttate, as is indicated in the table, was collected from a number of different plants both in the field and greenhouse and from the same plants on subsequent days. It was easily collected by sopping it up in glass wool and then squeezing it out into clean vessels. Chemical tests were made immediately and then the solution was quickly frozen to prevent any bacterial or mold action. These analyses are at best roughly quantitative and were made according to the method given by Morgan (15) for spot testing soil solution.[1] The total solids varied from approximately 2500 p.p.m. for No. 1 to approximately 600 p.p.m. for cabbage and tomatoes. Approximately 50 per cent of this material was of an organic nature.

The writer has advanced the hypothesis that the salts or compounds contained in the guttation fluid excreted from the host plant react or combine with the materials in sprays and dusts applied to plants as pesticides to form new compounds that either alter the effectiveness of the pesticide, or bring the copper into solution from the insoluble form from copper oxide and Bordeaux mixture. It has already been shown by Curtis (5) how guttation fluid is sucked back into the leaf. When this occurs, the soluble copper enters the plant to cause the well-known Bordeaux injury of Bordeaux-sensitive plants such as the cucurbits, or kill the leaf hopper (*Empoasca fabae* Herr) which causes hopper burn on potatoes.

It is a well-known chemical fact that one salt or compound has a very definite effect on the solubility of another. When plants that have been sprayed with Bordeaux mixture guttate, a whole series of inorganic salts in addition to unknown organic materials is brought directly in contact with the insoluble copper. An ideal condition is thus provided for the production of many new copper compounds. Tests were made to determine whether the guttation fluid could bring copper into solution from copper hydroxide. Ten ml. of distilled, tap, and guttation fluid from squash were agitated for 8 hours in flasks containing 100 mg. of copper hydroxide. When the supernatant solutions were filtered and tested,[2] no copper was found in either tap or distilled water, but 4 p.p.m. of copper were found in the solution containing guttation fluid. No copper was found in the guttation fluid as it came directly from the plant nor in either tap or distilled water.

INFLUENCE OF GUTTATION FLUID AND CERTAIN COMPOUNDS ON FUNGICIDAL EFFICIENCY

McCallan (12), Horsfall (11), and Dimond *et al.* (7), have described in detail methods for evaluating fungicides in the laboratory. A slight modi-

[1] The writer wishes to thank Miss Jane Andrews of the Soils Department of the Connecticut Agricultural Experiment Station for making these analyses.

[2] This test was made in the Department of Analytical Chemistry of the Connecticut Agricultural Experiment Station under the direction of Mr. W. T. Mathis.

fication of this method was used in determining the influence of guttation fluid on the toxicity of yellow copper oxide and Bordeaux mixture to *Macrosporium sarcinaeforme*.[3] This modification was: after the glass slides had been coated with the toxicants and dried, they were sprayed with two squeezes of a hand atomizer containing the guttation fluid and distilled water and held at constant level and distance from the slides. Table 2

TABLE 2.—*Effect of guttation fluid on copper toxicity*

Plants from which guttation water was collected	Spray time	Inhibition			
		Yellow copper oxide		Bordeaux mixture	
		Over sprayed with			
		Distilled water	Guttation fluid	Distilled water	Guttation fluid
	Seconds	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Summer squash growing in greenhouse, collected April 17, 1942, spore-tested April 17, 1942	40	100	100	90	100
	20	65	100	99	100
	10	46	100	89	100
	5	3	91	4	99
Tomatoes growing in the greenhouse, 12 weeks old, collected June 15, 1942, spore-tested Oct. 6, 1942	40	75	99	99	100
	20	51	94	91	99
	10	41	83	46	65
	5	28	66	8	23
Tomatoes in greenhouse, 10 weeks old, collected May 12, 1942, spore-tested May 12, 1942	40	.....	100	81	99
	20	47	93	50	95
	10	19	95	22	65
	5	11	28	10	7
Same as above except that the plants were fertilized with NH_4SO_4 on May 6, 1942	40	.....	91	81	89
	20	47	71	50	80
	10	19	12	22	9
	5	11	6	10	2
Cucumbers growing in the greenhouse, collected Aug. 13, 1942, spore-tested Oct. 6, 1942	40	73	100	99	99
	20	51	99	91	98
	10	41	95	46	79
	5	28	86	8	19
Same plants as above except guttate was collected Aug. 13, 1942	40	73	92	99	99
	20	51	96	91	98
	10	41	85	46	88
	5	28	70	8	41
Cabbage growing at the Exp. Station farm, Mt. Carmel, collected July 19, 1942, spore-tested Oct 6, 1942	40	73	100	99	100
	20	51	84	91	98
	10	41	59	46	59
	5	28	44	8	19
Summer squash growing at the Exp. Station farm, Mt. Carmel, collected July 19, 1942, spore-tested Oct. 6, 1942	40	73	88	99	99
	20	51	75	91	93
	10	41	66	46	67
	5	28	31	8	21

and figure 1[4] give the results of these tests, which show clearly that the guttation fluid increased to a marked degree the per cent of inhibition of spore germination. In figure 1 not only are all the lines from the guttation fluid series parallel to each other, but their slope is also different from the

[3] Thanks are given to Dr. James G. Horsfall, Dr. Albert Dimond and Miss Florence McWilliam of the Department of Botany and Plant Pathology of the Connecticut Agricultural Experiment Station for making these spore tests.

[4] The advantages of plotting these data on Logarithmetic Probability paper and the L.D. 50 values referred to are discussed in detail by Dimond *et al.* (7).

distilled water check. This difference in slope shifts the L.D. 50 values decidedly to the left, thus indicating that the guttation fluid brought more copper into solution to inhibit spore germination than did distilled water. These results support the hypothesis presented by the writer in the preced-

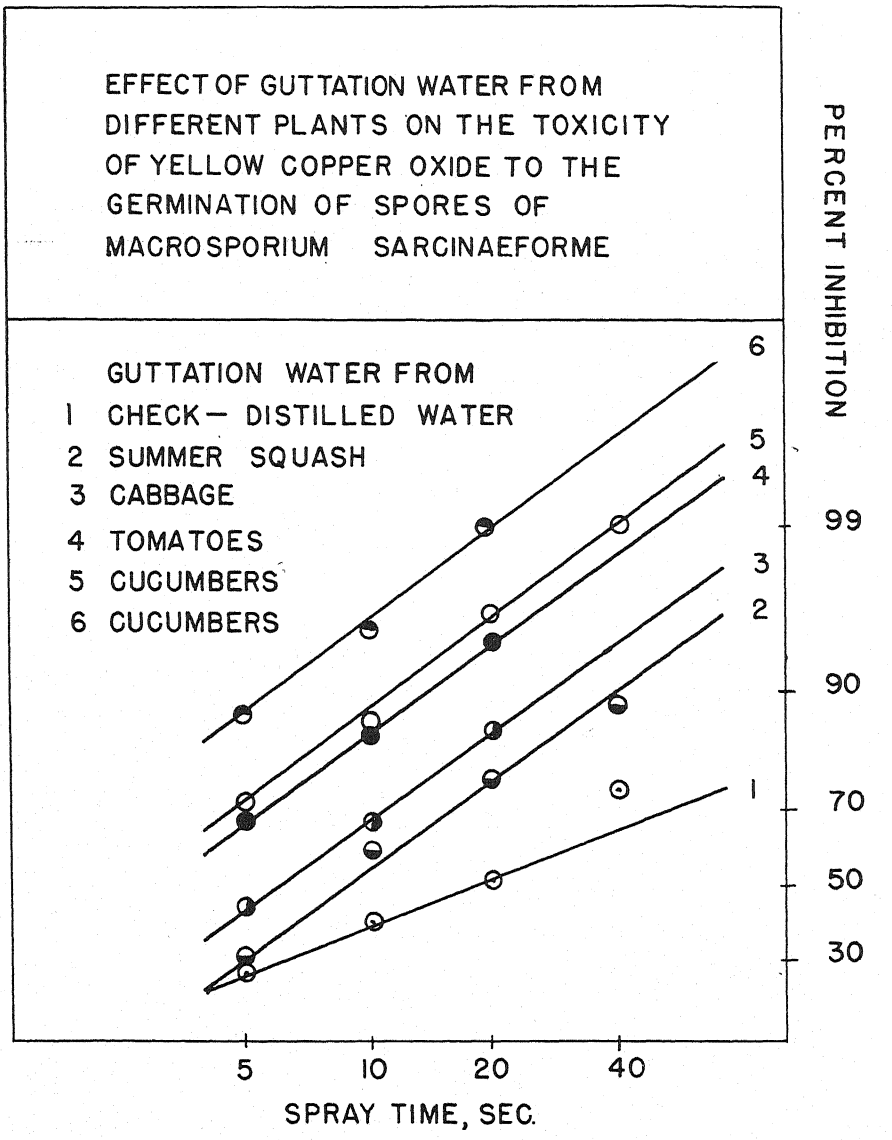


FIG. 1

ing paragraphs. Furthermore, the parallel lines in figure 1 indicate that the guttation fluid from different species of plants affect the copper oxide in the same way but at different rates. This fact presents the question: is there some one compound common to the guttation fluid from all plants, but

present in different amounts? Since ammonia readily forms complex compounds with copper, perhaps the ammonia was the most likely compound to give this effect. The results of a preliminary experiment in which 20, 10, and 5 p.p.m. of ammonium chloride and ammonium hydroxide tested in the same way as the guttation fluid, are given in table 3. The ammonium

TABLE 3.—*Effect of NH_4CL and NH_4OH on copper toxicity*

Chem. compound and p.p.m.	Spray time	Inhibition			
		Yellow copper oxide		Bordeaux mixture	
		Over sprayed with			
		Distilled water	Salt solution	Distilled water	Salt solution
	Seconds	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ammonium chloride					
20 p.p.m.	40	95	100	98	100
	20	85	100	86	94
	10	52	97	33	61
	5	10	60	9	12
10 p.p.m.	40	95	100	98	95
	20	85	99	86	95
	10	52	88	33	47
	5	10	38	9	12
5 p.p.m.	40	95	100	98	98
	20	85	97	86	93
	10	52	93	33	56
	5	10	62	9	11
Ammonium hydroxide					
20 p.p.m.	40	94	99	.....	.....
	20	82	91	.....	.....
	10	51	61	.....	.....
	5	12	16	.....	.....
10 p.p.m.	40	94	96	.....	.....
	20	83	94	.....	.....
	10	51	37	.....	.....
	5	12	19	.....	.....
5 p.p.m.	40	94	96	.....	.....
	20	82	85	.....	.....
	10	51	40	.....	.....
	5	12	10	.....	.....

chloride increased the per cent of inhibition of spore germination above the distilled water check on both yellow copper oxide and Bordeaux. While the difference between the ammonium hydroxide and distilled water on yellow copper oxide is less pronounced, there is, nevertheless, an increased inhibition.

A second experiment was made in which 6 salts, ammonium citrate, ammonium chloride, ammonium sulphate, sodium citrate, potassium chloride, and potassium sulphate were used in concentrations of 20 and 10 p.p.m. The results (Table 4) show that all the salts increased the per cent of inhibition of spore germination over that of distilled water. On the yellow copper oxide the ammonium salts had a greater effect than either sodium or potassium, while there was little difference on the Bordeaux series between any of the salts.

TABLE 4.—*Effect of different salts on copper toxicity*

Chem. compound and p.p.m.	Spray time	Inhibition			
		Yellow copper oxide		Bordeaux mixture	
		Over sprayed with			
		Distilled water	Salt solution	Distilled water	Salt solution
	Seconds	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ammonium citrate					
20 p.p.m.	40	19	53	90	99
	20	13	31	78	82
	10	5	11	16	29
	5	5	7	6	8
10 p.p.m.	40	19	70	90	99
	20	13	18	78	82
	10	5	7	16	16
	5	5	4	6	5
Ammonium chloride					
20 p.p.m.	40	19	93	90	97
	20	13	74	78	62
	10	5	21	16	31
	5	5	8	6	8
10 p.p.m.	40	19	62	90	98
	20	13	34	78	78
	10	5	14	16	30
	5	5	6	6	9
Ammonium sulphate					
20 p.p.m.	40	19	67	90	99
	20	13	12	78	87
	10	5	6	16	19
	5	5	2	6	12
10 p.p.m.	40	19	50	90	92
	20	13	13	78	69
	10	5	4	16	11
	5	5	1	6	3
Sodium citrate					
20 p.p.m.	40	19	35	90	97
	20	13	15	78	86
	10	5	7	16	21
	5	5	5	6	5
10 p.p.m.	40	19	23	90	98
	20	13	13	78	70
	10	5	7	16	34
	5	5	2	6	6
Potassium chloride					
20 p.p.m.	40	19	33	90	99
	20	13	8	78	77
	10	5	5	16	16
	5	5	1	6	4
10 p.p.m.	40	19	44	90	.....
	20	13	8	78	66
	10	5	5	16	24
	5	5	1	6	4
Potassium sulfate					
20 p.p.m.	40	19	43	90	94
	20	13	10	78	67
	10	5	4	16	33
	5	5	5	6	8
10 p.p.m.	40	19	22	90	88
	20	13	11	78	64
	10	5	4	16	35
	5	5	1	6	5

Baine (1) lists $CaCl_2$, $Ca(NO_3)_2$, NaCl, and $Mg(NO_3)_2$ as having materially increased Bordeaux injury to peach leaves while KNO_3, cane and grape sugar were less effective, $Ca_3(PO_4)_2$, K_3PO_4 were indifferent, and KH_2PO_4 seemed to diminish the injury. It would be difficult to determine which or how much of the compounds Baine (1) used in this experiment, or those of the writer, occur in the guttation fluid. From the results of these experiments, however, there can be little doubt that the compounds in solution in the guttation fluid must at times play an important part in bringing copper into solution from the insoluble form to increase its effectiveness as a fungicide, and that the ammonia in this fluid must be an important constituent in bringing about this effect. Other compounds, either singly or collectively, undoubtedly have similar effects.

Crandall (4) was probably, at least in part, correct in his statement about the ammonia contained in the rain having a solubilizing effect on the copper fungicide. Failyer and Willard (8) have shown that rain water, especially in amounts of less than 2.5 millimeters, contains appreciable amounts of ammonia. In one such rain they reported 12.2 p.p.m. of nitrogen. According to the experiments described above this would certainly be sufficient amount to bring copper into solution from Bordeaux.

THE RELATION BETWEEN GUTTATION FLUID AND SPRAY INJURY

A good many observations have been made by different investigators as well as fruit and vegetable growers concerning the injury to certain plants following the use of Bordeaux mixture or other sprays or dusts. There seems to be an unanimity of opinion among them that this injury is nearly always associated with wet, foggy weather or conditions of high temperature and humidity. Frequent reference, Baine (1), Crandall (4), also is made to heavy dews and meteoric waters on the sprayed foliage, which later shows injury. Furthermore, they are universally agreed that the copper must enter the leaf to cause injury, yet its method of entry is still a controversial subject. DeLong (6) has given an excellent review of most of the literature on this subject.

In only one of these observations, as far as the writer has been able to determine, has guttation fluid been collected and its solubilizing effect determined on a spray or dust. Smith (16) collected the "dew" from cotton plants and found that it increased markedly the solubility of calcium arsenate. He attributed this effect to the chemical action of the compounds in the dew on the calcium arsenate. Unquestionably, Smith, in this work, found the most important factor that was responsible for the increased solubility of calcium arsenate when it was applied to cotton as an insecticide. He concluded that the soluble arsenate is responsible for the injury of this dust to the plant. Although the title of this paper is "Excretions from Leaves as a Factor in Arsenical Injury to Plants," he was uncertain how these compounds got into the dew. He says: "Whether these have come into the dew by osmosis or by actual exudation was not determined, the for-

mer being more probable." In discussing the influence of various chemical compounds, found as impurities in the insecticide or in the water with which spray and dusts are mixed, on the solubility of arsenicals he makes this statement: "Their work suggests that the burning of di-lead arsenate frequently observed on the Pacific Coast, and hitherto attributed to hydrolysis by the heavy and recurring fogs of that region, is really due to the salt spray entrapped by these fogs." It seems to the writer that there was little need of looking for an outside agency in supplying chemical impurities when, under foggy conditions the plant probably would guttating to supply them.

Baine (1) made some pertinent statements concerning the entrance of copper into the peach leaf. In every instance in which he refers to this he emphasized the necessity of moisture being present on the leaf. ". . . it may be true that there is sufficient moisture in the cuticle of the living leaf to allow for the diffusion of a minute quantity of copper into the leaf." In another paragraph in which he discusses his experiments with deliquescent copper salts on peach leaves he states, "Here the salts absorb moisture from the rapidly cooling air in the afternoon and become a saturated solution just at the most favorable time for its entrance into the leaf by imbibition." He was not sure whether the copper entered by diffusion through the cuticle or by imbibition. Where deliquescent copper salts were used he was certain that it entered by imbibition. This action he described as occurring especially toward evening when the dew begins to fall during showery weather.

In the writer's opinion Baine, Smith, and Crandall all observed the solubilizing influence of guttation water on Bordeaux mixture, and much of the meteoric water and dew which they discuss was actually fluid excreted or guttated by the plant.

It is possible that Baine actually saw the leaf imbibe water in the same way that the writer, Curtis (5), has seen and demonstrated that the guttation fluid is sucked back into the leaf. The writer is certain, however, that Baine did not see dew "fall" in the evening.

SUMMARY

Chemical analyses of guttation fluid collected from tomatoes, cabbage, cucumbers, summer and Hubbard squash showed that the water contains in solution both organic and inorganic salts of varying concentrations and pH, that the solutes from the same plants may vary from day to day, and that the concentration of the solutes may be influenced by soil fertilization.

The guttation fluid from squash plants was shown to bring copper into solution from $Cu(OH)_2$; furthermore, the guttation fluid from all the plants tested increased the toxicity of yellow copper oxide and Bordeaux mixture when sprayed on glass slides and seeded with known dilutions of *Macrosporium sarcinaeforme*. Different concentrations of several salts gave similar effects.

The hypothesis advanced to explain how sprays and dusts applied as pesticides injure the plants is that the guttation fluid increases the solubility of the pesticide, which is then sucked back into the leaf in the guttation drops so that injury occurs from the inside of the leaf. This hypothesis can explain also the process by which on plants previously sprayed with Bordeaux the copper content of potato leaves can be built up in concentrations toxic to the potato leaf hopper, *Empoasca fabae* (Herr).

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THE PERFECT STAGE OF COLLETOTRICHUM FALCATUM

FERNANDO CARVAJAL AND C. W. EDGERTON

(Accepted for publication July 2, 1943)

The fungus causing the red rot of sugar cane, *Colletotrichum falcatum,* was first described from Java by Went in 1893 (6). In the imperfect stage, the only stage known until 1942, falcate, unicellular, hyaline conidia develop in acervuli on conidiophores, which are intermixed with numerous setae. The conidia form on short conidiophores and, sometimes, even on the setae. The conidia upon germination may either develop a mycelium directly or produce appressoria similar to those produced by certain other members of the order Melanconiales. The fungus fruits on various parts of the sugar-cane plant, but the acervuli are particularly common on the midribs of the leaves. The fungus occurs in all sugar-producing countries, and, in some, causes serious losses to the cane industry. In Louisiana it has been known since 1908 (3, 4) and is recognized as one of the most serious and important disease-producing organisms on sugar cane.

In 1942, while studying the red-rot fungus on some cane leaves that had been sterilized in the autoclave and inoculated with a pure culture, a perithecial stage, later identified as belonging to the genus *Physalospora,* was observed. This ascigeral stage has since been found very commonly prevalent in the field under certain conditions, and has been produced in the laboratory on cane leaves, either sterilized or not, and on other fibrous material. It has been proved to be the perfect or ascigeral stage of *Colletotrichum falcatum.*

OCCURRENCE OF PERITHECIA ON CANE LEAVES

In the field the perithecia have been seen most commonly on dead leaf blades and leaf sheaths that were beginning to dry. Leaves on shoots killed by too much crowding or from other causes frequently have been found covered with perithecia. It also has been possible usually to hasten perithecial formation by cutting leaves showing midrib lesions and placing them on moist cotton in moist chambers. Generally, as the tissues have lost their chlorophyll and died, the perithecia have developed and matured. The perithecia have occurred in abundance on the leaf sheaths, on the underside of the midrib, and on both surfaces of the rest of the leaf blade.

The perithecia generally have been found under field conditions somewhat later than the period of greatest conidial production; and this, combined with the fact that they have not been very conspicuous, is apparently the reason why they have not been reported previously.

Perithecia have been collected in Louisiana on plants of the 5 recognized species of *Saccharum, S. officinarum* L., *S. barberi* Jeswiet, *S. sinense* Roxb., *S. spontaneum* L., and *S. robustum.* They also have been collected on leaves of the grass *Leptochloa filiformis* (Lam.) Beauv., which is very common in

sugar-cane fields in Louisiana. They also have been collected on numerous hybrids of the above cane species. There is no reason to doubt their development on all cane varieties.

In the laboratory, perithecia have developed not only on diseased cane leaves brought in from the field, but also upon sterilized leaves of cane, sorghum, and corn placed on wet cotton in Petri dishes, and even on sterilized strips of filter paper placed on cotton wet with a nutrient solution in Petri dishes following inoculation with a pure culture of the red-rot fungus.

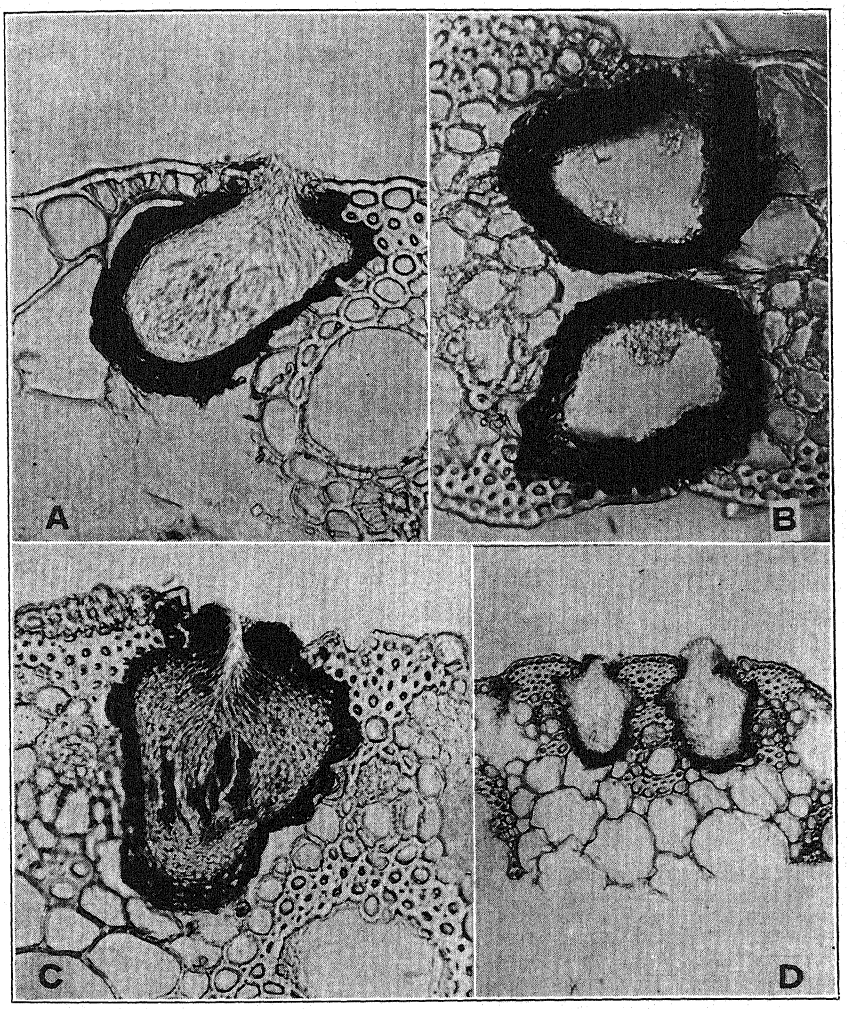


FIG. 1. Perithecia of *Physalospora tucumanensis*. A. Perithecium from upper surface of leaf. ×240. B. Perithecia on both surfaces of leaf. ×240. C. Perithecium showing usual position between fibrovascular bundles. ×240. D. Two perithecia closely associated with fibrovascular bundles. ×120.

STRUCTURE OF PERITHECIA

On a leaf the perithecia normally develop between fibrovascular bundles, filling all the available space (Fig. 1). In a cross section of a leaf the peri-

thecia may appear to be quite irregular due to the perithecial wall following the contours of the bundles. In a longitudinal direction, with no restricting bundles, the perithecia are usually considerably wider and the walls are rounded (Fig. 2, D). The perithecia are almost entirely under the surface with only a very small portion of the ostiole outside the epidermis. At maturity the perithecia are filled with a large number of asci and numerous paraphyses (Fig. 3). The paraphyses, which extend to the ostiole, are deli-

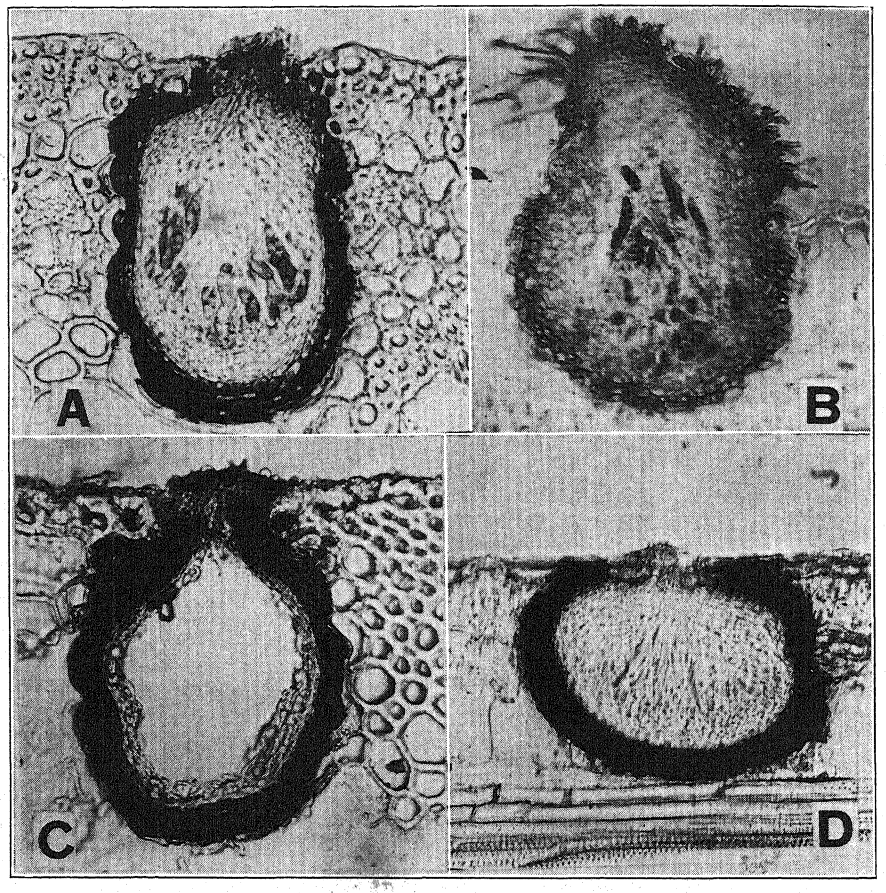


FIG. 2. Perithecia of *Physalospora tucumanensis*. A. Perithecium with asci. ×300. B. Perithecium from autoclave-sterilized leaf inoculated with red-rot culture. ×240. C. Perithecium from type material from Argentina. ×300. D. Young perithecium, section cut parallel to long axis of leaf. ×240.

cate structures. In a smear preparation, their walls are difficult to see, but the abundant granules or oil drops in the interior are very conspicuous. The asci are club-shape and somewhat thickened at the apices. The ascospores are elliptical, hyaline, unicellular, straight or slightly fusoid, each containing, like the conidia of *Colletotrichum falcatum*, a central clear area, which is assumed to be a nucleus.

Perithecia that develop on sterilized leaves are larger, more thin-walled, and irregular, and project farther from the surface than those on leaves from the field (Fig. 2, B).

ISOLATION AND INOCULATION TESTS

To study the *Physalospora* from sugar cane, and to prove beyond question its relation to the red-rot fungus, cultures were obtained from ascospores from various cane varieties and from all the cane species and the grass *Leptochloa filiformis*. In the course of the study, at least 497 pure cultures were obtained from single ascospores from perithecia that developed naturally on leaves. These were picked up with the micromanipulator, germi-

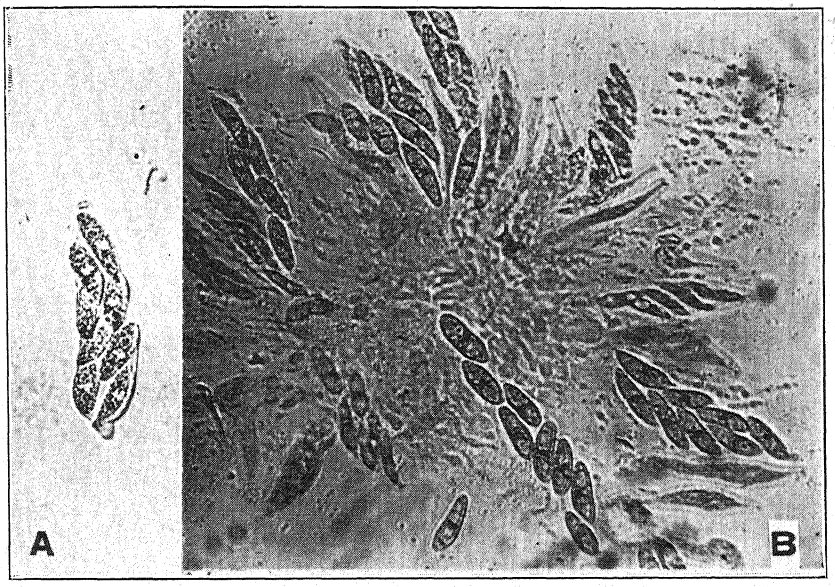


FIG. 3. Asci of *Physalospora tucumanensis*. A. Single ascus, not stained, showing light-colored area in center of each spore. ×400. B. Mass of asci from a perithecium, stained with Cotton Blue. ×400.

nated, and transferred to culture tubes. These cultures grew rapidly and produced in abundance falcate conidia typical of *Colletotrichum falcatum*. In no way did these cultures differ from those obtained from conidia of the red-rot fungus. Cultures of both the light and dark strains, as described by Abbott (1), were obtained from single ascospores. Of the 497 cultures studied, 357 were of the dark strain or similar to it, while 140 were of the light strain. In some cases both strains were obtained from the same midrib or leaf sheath material, though in no case did both strains come from the same perithecium.

To prove with certainty that the *Physalospora* found on sugar cane was the perfect stage of the red-rot fungus, *Colletotrichum falcatum*, numerous inoculation experiments were made on sugar cane with conidia that de-

veloped in single ascospore cultures. In all, more than 50 different cultures were used in the tests. Both leaves and stalks were inoculated.

Leaves in the field were inoculated by inserting conidia in punctures on the upper surface of the midrib. Three leaf inoculation tests were carried on at various times during the summer, using a number of different ascospore cultures along with known red-rot cultures. The lesions produced on the inoculated leaves were in all ways similar to lesions produced by virulent red-rot cultures. They were elongated, reddish to brownish, usually with a

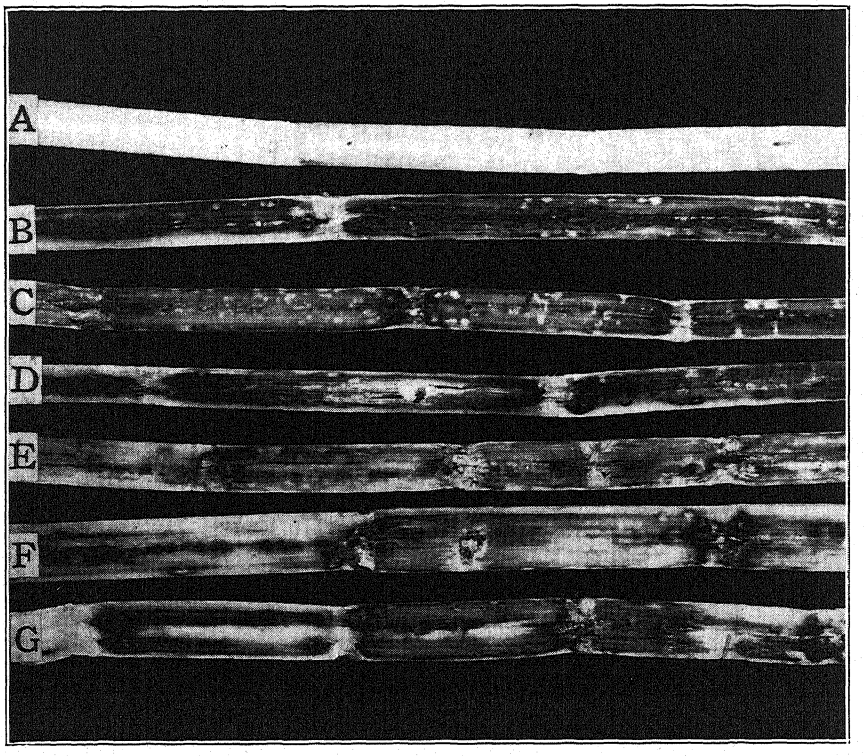


FIG. 4. Red rot in stalks of cane following inoculation with single-spore cultures of *Colletotrichum falcatum* and *Physalospora tucumanensis*. A. Control, not inoculated. B. Co. 281 inoculated with single-ascospore culture. C. Co. 281 inoculated with single-conidium culture of *Colletotrichum falcatum*. D. Co. 281 inoculated with single-ascospore culture isolated from a leaf that had been inoculated with a culture of *Colletotrichum falcatum* that had been carried through 40 generations by conidial transfers. E. Co. 281 inoculated with single-ascospore culture. F, G. C.P. 33/243 inoculated with different single-ascospore cultures.

light-colored central area on which the acervuli developed in abundance. Many of the inoculated leaves showing lesions were cut and placed in moist chambers. As the green color faded after several days, perithecia developed in abundance on them.

The inoculations in the stalks were of 3 types, these being similar to the routine tests made in testing seedlings and new canes for resistance to red rot. These included inoculations in standing cane in the field, inoculations

in cut stalks kept in the laboratory, and inoculations in cut stalks just previous to planting in the field. All stalk inoculations were made by inserting spores in the stalks in holes made with a metal inoculator, a cutting instrument that removes a plug of stalk tissues.

Inoculations in the field were made at various times using single ascospore cultures along with known virulent red-rot cultures. In such tests the conidia migrated up and down in the fibrovascular bundles. Red lesions developed along the bundles wherever the spores lodged and germinated.

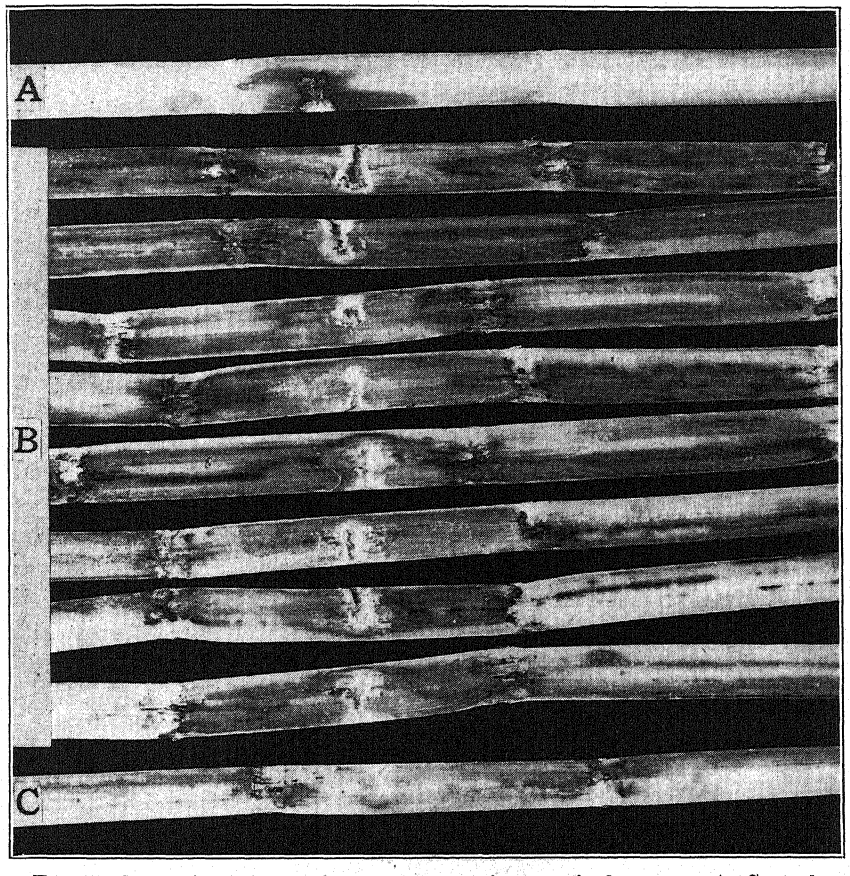


FIG. 5. Test with cultures from ascospores from a single ascus. A. Control, not inoculated. B. Stalks inoculated with cultures from 8 ascospores from one ascus. C. Stalk inoculated with single-ascospore culture isolated from a leaf that had been inoculated with a culture of *Colletotrichum falcatum* that had been carried through 40 generations by conidial transfers.

Many ascospore cultures were tested and all produced the same typical red lesions, as did the red-rot cultures.

Inoculations with many cultures also were made in cut stalks stored in the laboratory. These all produced the more generalized type of injury characteristic of stalks in this condition.

In stalks planted in the fall after inoculation, the mycelium spread through the stalks during the fall and winter months. The rot produced by

ascospore cultures was identical with that produced by virulent red-rot cultures (Fig. 4).

Numerous cultures were made from the inoculated leaves and stalks and in all cases typical red-rot cultures were obtained.

HOMOTHALLISM OF PHYSALOSPORA

The inoculation tests and culture experiments also have demonstrated that the sugar-cane *Physalospora* is homothallic. It always has been possible to force the formation of perithecia on leaves or leaf sheaths inoculated with single ascospore cultures. Also, perithecia have been produced in abundance on sterilized leaves and other materials inoculated with such cultures.

RESULTS WITH ASCOSPORES FROM SAME ASCUS

In several of the experiments with the sugar-cane *Physalospora,* it was possible to obtain pure cultures from all 8 ascospores from a single ascus. In all cases these cultures were absolutely identical as far as could be determined. Furthermore, these were used in inoculation tests, and the same symptoms were produced by all cultures (Fig. 5).

DETERMINATION OF LOUISIANA FUNGUS

In the attempts to determine the *Physalospora* on sugar cane in Louisiana, its characters were compared with those of similar fungi reported on sugar cane and related plants in various parts of the world. Of the several species of *Physalospora* reported on sugar cane, the characters of one, *P. tucumanensis* Speg., seemed to agree closely with those of the Louisiana material. This fungus was first described in 1896 in Argentina by Spegazzini (5), who found it occurring commonly on dying leaves of sugar cane. In order to determine with reasonable certainty whether or not the Louisiana fungus was the same as that from Argentina, a fragment of the type material of Spegazzini's fungus, *Physalospora tucumanensis,* was obtained from Argentina.[1]

This fragment contained perithecia (Fig. 2, C) in abundance and also setae and conidia of what seemed to be *Colletotrichum falcatum.* Upon examination, it was found that the perithecia, asci, and ascospores were very similar to those of the fungus found in Louisiana, which had been proved to be the perfect stage of *Colletotrichum falcatum.* With this evidence, the Louisiana fungus was reported as *Physalospora tucumanensis* Speg. (2).

DESCRIPTION OF P. TUCUMANENSIS

The characteristics of *Physalospora tucumanensis* based on studies of Louisiana collections are given in the following description.

Physalospora tucumanensis Speg. Perithecia mostly scattered, sometimes 2 or 3 together, on various parts of host plant but abundant on leaf sheath and leaf blade, nearly submerged with only a small portion of ostiole protruding, located usually between fibro-

[1] Type material was kindly furnished by Juan C. Lindquist of the Instituto de Botanica "Spagazzini," La Plata, and was labeled "No. 418, separado del tipo."

vascular bundles and often filling entirely the space between the bundles, black, 100–260 (width) × 85–250 (height) μ in size, the width in longtudinal section of leaf greater than in cross section. Asci numerous, clavate, thickened at the apex, 50–118 × 7.4–19.2 μ, usually ranging from 70–90 × 13–18 μ. Ascospores irregularly biseriate, single-celled, hyaline, straight or somewhat fusoid, elliptical to ovate, at maturity and, while fresh, containing a hyaline area near center, 12.5–30.0 × 5.0–11.1 μ, usually ranging from 18–22 × 7–8 μ. Paraphyses very abundant, delicate walls, septate, usually unbranched, filled with conspicuous granules or oil droplets, extending to ostiole. Periphyses abundant and conspicuous around ostiole opening.

Found in abundance on dead and dying leaves of species of *Saccharum* and *Leptochloa filiformis* in Louisiana, usually following conidial formation of *Colletotrichum falcatum*. Proved to be the ascigeral stage of *Colletotrichum falcatum* Went.

SUMMARY

A fungus belonging to the genus *Physalospora* has been found in abundance on dead and dying leaves and other parts of species of *Saccharum* and *Leptochloa filiformis* in Louisiana usually following the production of conidia of *Colletotrichum falcatum*.

By inoculation tests, the *Physalospora* has been proved to be the ascigeral stage of *Colletotrichum falcatum*, the fungus causing the red rot of sugar cane.

The perithecia have been produced in the laboratory on sterilized leaves and other fibrous material.

The *Physalospora* is homothallic, as perithecia have been readily produced with single ascospore cultures.

The 8 ascospores from an ascus have produced identical cultures with the same pathogenicity.

The fungus has been identified as *Physalospora tucumanensis* Speg., a species collected in Argentina by Spegazzini and described by him in 1896. The identification has been confirmed by comparing the Louisiana material with type specimens of *P. tucumanensis*.

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FURTHER STUDIES ON A SPECIES OF HELMINTHOSPORIUM PARASITIZING CORN[1]

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INTRODUCTION

In 1941 (6) a species of *Helminthosporium* was reported occurring as a parasite on dent corn (*Zea mays* L.) in the Corn Belt. The species was shown to be composed of two physiological races, morphologically indistinguishable, but separable on the basis of symptoms each produced and the high degree of specialization in parasitism of one of the races toward certain inbred lines of corn. Susceptibility to this race was shown to be inherited as a monogenic recessive (7).

Morphological comparisons between the species reported and that of *Cochliobolus heterostrophus* Drechs. (*Helminthosporium maydis* Nisikado and Miyake) as found in the literature (1, 2, 3, 4), indicated close agreement in range of spore length, range of spore width, number of septations, character of the hilum, mode of spore germination, and range in length of conidiophores. Symptoms on mature leaves also bore similarity to those caused by *C. heterostrophus,* as shown in illustrations (2), and as seen on examination of herbarium material. The fungus under consideration was identified as *H. maydis* because of these similarities in morphology and in symptoms it produced.

At the time the study was being made, attempts to obtain viable collections of *Helminthosporium maydis* from the Southern States for comparison were unsuccessful. In the late summer of 1942, a relatively severe epidemic of leaf spot of corn caused by *H. maydis* occurred in the Southeastern States (8). Through the courtesy of George Y. Young several collections of *H. maydis* were obtained for comparison with the species occurring in the Corn Belt states.

OBSERVATIONS

Pure cultures of *Helminthosporium maydis* were obtained from 4 collections made in Mississippi and from 1 collection made in North Carolina. At the same time pure cultures were made from collections of the species occurring in the Corn Belt. Of the latter cultures, 3 were of race I and 3 of race II. Spore suspensions of each were sprayed on seedlings of inbred lines, single crosses, and double crosses. The seedlings were placed in moist chambers overnight and then returned to the greenhouse bench. Care was taken to prevent any mixing of inoculum before and after spraying on the

[1] Cooperative Investigation, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station. Journal Paper No. 116 of the Purdue University Agricultural Experiment Station.

[2] Associate Pathologist, Division of Cereal Crops and Diseases.

plants. One week after inoculation, when symptoms were well established, the results were recorded as shown in table 1. These data indicate no specialization in parasitism among the southern isolates for the inbred lines and hybrids inoculated. Tests were continued to include 57 inbred lines, 8 single crosses, and 6 double crosses. All material tested showed equal susceptibility to all isolates from southern collections.

The symptoms produced by races I and II on seedling leaves (Fig. 1, A), as well as on mature plant leaves, are distinct from each other and have already been described (6). The symptoms produced by the southern iso-

TABLE 1.—*Reaction of inbred lines, single crosses, and double crosses to inoculation with isolates of Helminthosporium sp. from Corn Belt collections, and with isolates of H. maydis collected in the Southern States*

Inbred or hybrid inoculated	Isolates used for inoculation										
	Isolates of the Corn Belt species						Isolates of *H. maydis*[d]				
	Race I			Race II			Mississippi				N.C.
	(K61)[a]	(K44)	(Pr)	(P8)	(187–2)	(WF9)	No. 1	No. 2	No. 3	No. 4	No. 1
Inbreds											
K61	S[c]	S	S	M.S.	M.S.	M.S.	S	S	S	S	S
K44	S	S	S	"	"	"	"	"	"	"	"
Pr	S	S	S	"	"	"	"	"	"	"	"
Pr 1[b]	R	R	R	"	"	"	"	"	"	"	"
187–2	R	R	R	"	S	"	"	"	"	"	"
38–11	R	R	R	M.R.	M.R.	M.R.	"	"	"	"	"
Single crosses											
K61 × Pr	S	S	S	M.S.	M.S.	M.S.	"	"	"	"	"
K61 × K44	S	S	S	"	"	"	"	"	"	"	"
187–2 × L317	R	R	R	"	"	"	"	"	"	"	"
Double crosses											
U. S. 13	R	R	R	"	"	"	"	"	"	"	"
Ind. 844	R	R	R	"	"	"	"	"	"	"	"

[a] Designations in parentheses are of inbred lines from which isolates were obtained.
[b] Homozygous resistant inbred Pr obtained by outcrossing to a resistant inbred, backcrossing five successive generations, and selfing twice.
[c] S. = Susceptible; R. = Resistant; M.S. = Moderately susceptible; M.R. = Moderately resistant.
[d] From the Southern States.

lates on seedling leaves, while resembling those caused by race I, are nevertheless distinct (Fig. 1, B). In the early stages of development of the lesions, water soaking of the tissues is a conspicuous symptom. Young lesions produced by race I, while they do show water soaking, do not do so to the extent found in infections caused by *H. maydis* from southern collections. As compared with symptoms of race I, fully developed lesions on seedling leaves caused by the southern isolates are somewhat more elongated and the margins of the lesions are more irregular. A definite zonate pattern is characteristic of the lesions of both diseases. In those infections caused by the southern isolates a purplish-brown margin surrounding the dry straw-colored area is a distinct feature.

Morphological Comparisons

Spore suspensions of the southern isolates and of race I and of race II of the Corn Belt species were separately sprayed on seedlings of K61 × Pr, a single cross susceptible to all cultures. Ten days after inoculation individual collections of seedling leaves, each of which was infected with a single

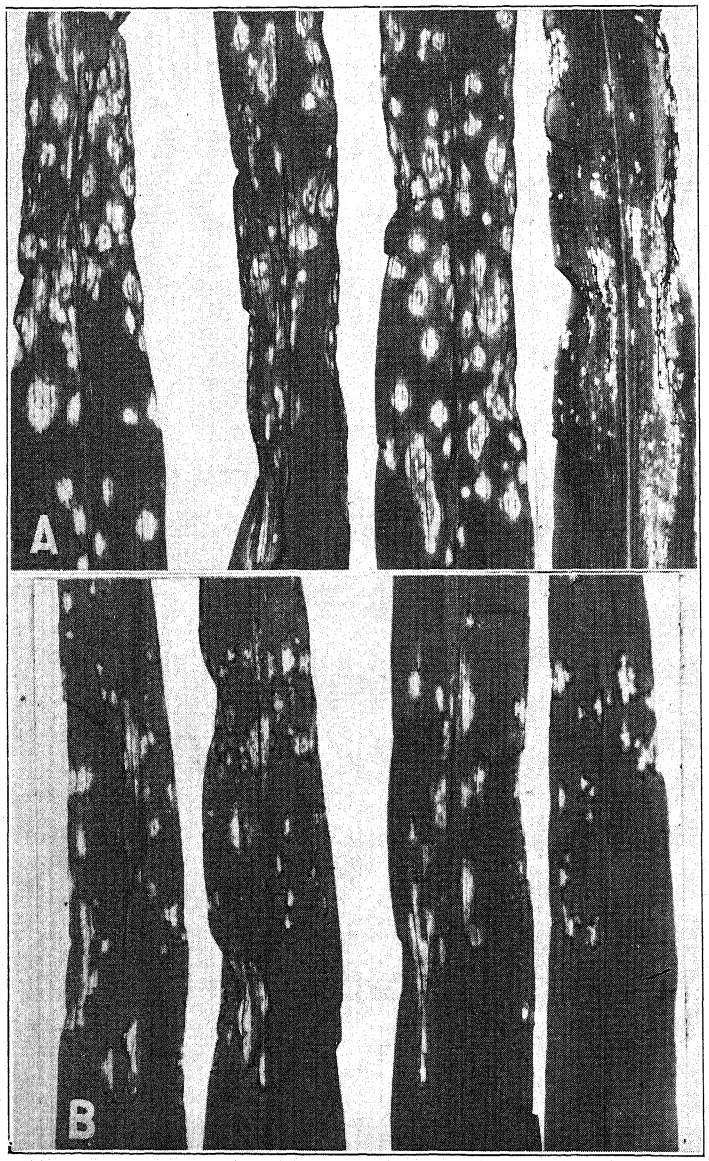


FIG. 1. A. Symptoms on seedling leaves of K61 × Pr, produced by isolates of *Helminthosporium carbonum*. The first three leaves infected with isolates of race I, the fourth leaf infected with an isolate of race II. B. Symptoms on seedling leaves of K61 × Pr produced by isolates of *H. maydis* collected in the Southern States.

culture, were harvested and dried at room temperatures for 2 weeks. The dried leaves were then placed in moist chambers for 4 days at which time spores had been produced in abundance on the lesions. Spores were scraped at random from lesions scattered over the leaves and spore length, spore width, and number of septations recorded. Three samplings totaling 105 spores were made from each culture. The data obtained are shown in table 2. Data for spore length of the southern collections are compared with

TABLE 2.—*Comparative measurements of spores grown from collections of Helminthosporium maydis and H. carbonum on seedling leaves of the susceptible single cross K61 × Pr*

Culture No.	Range of spore length	Mean spore length	Range of spore width	Mean spore width	Range in number of septa	Mean number of septa
			Southern collections (*H. maydis*)			
	Microns	*Microns*	*Microns*	*Microns*		
1	42.5–127.4	86.7	10.6–21.2	15.2	5–11	8.1
2	35.4–120.4	92.9	7.1–17.7	15.7	3–13	8.5
3	24.8–120.4	91.8	10.6–17.7	14.3	3–12	8.5
4	31.9–123.9	85.5	10.6–17.7	14.4	3–11	8.2
5	46.0–127.4	89.1	10.6–17.7	14.6	3–12	8.2
Grand means		89.2		14.9		8.3
			Northern collections (*H. carbonum*)			
(Race I)						
6	38.9– 99.1	69.5	10.6–14.2	13.5	3–12	7.2
7	31.9– 99.1	56.9	10.6–17.7	13.0	3–10	6.4
8	24.8– 95.6	57.1	7.1–17.7	12.4	2–11	6.8
Means for race I		61.2		13.0		6.8
(Race II)						
9	24.8–102.7	65.9	10.6–14.2	12.7	3–12	7.7
10	35.4–102.7	65.2	10.6–17.7	14.3	3–11	6.9
11	31.9– 88.5	60.9	10.6–17.7	13.6	3–11	7.0
Means for race II		64.0		13.5		7.2
Grand means		62.6		13.3		7.0

those for the northern collections by means of frequency polygons in figure 2, A. In figure 2, B, the spore length distributions of race I and race II of the northern collections are illustrated.

The difference of 26.6 μ between the mean spore lengths of the northern and the southern collections is statistically significant. The difference of 2.8 μ between mean spore lengths of race I and race II is not statistically significant.

Comparisons between the spores of the northern collections with those of the southern collections in respect to such characters as color and curvature emphasize further differentiation. These differences are illustrated in figure 3. Spores from southern collections show considerable curvative as compared with the relatively straight spores from collections of the Corn

Belt species. Spores from southern isolates are fuliginous to olivaceous in color, whereas those from the Corn Belt species are dark olivaceous-brown. All comparisons were made on material of the same age collected from infected leaves of one single cross susceptible to all isolates.

With the exception of *Helminthosporium maydis* Nisik. and Myke. and *H. zeicola* Stout, the species of *Helminthosporium* under consideration bears little resemblance to any of the other species found on corn or other grass hosts. The differences between it and *H. maydis* as pointed out in this paper

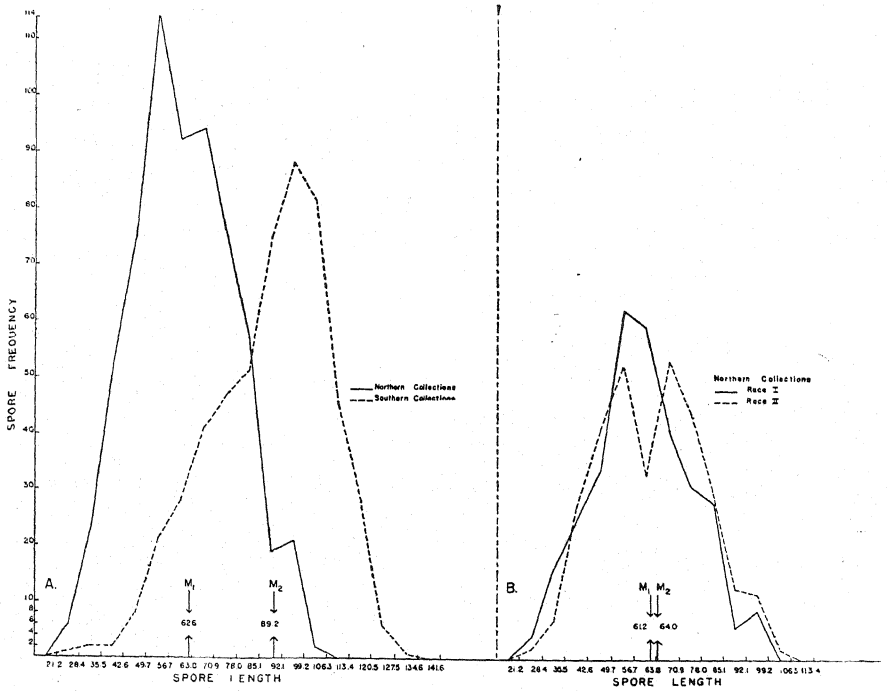


FIG. 2. A. Frequency distribution of spore length of northern collections (*Helminthosporium carbonum*) compared with that of southern collections (*H. maydis*). M_1 (62.6 μ) = mean length of spores from northern collections. M_2 (89.2 μ) = mean length of spores from southern collections. The difference between these means is statistically significant. B. Frequency distribution of spore length of race I and race II of northern collections (*H. carbonum*). M_1 (61.2 μ) = spore length of race I. M_2 (64.0 μ) = mean spore length of race II. Difference between M_1 and M_2 is not significant.

appear sufficient to set it apart as a distinct species. *H. zeicola* Stout, was described in 1930 (5) and similarities between it and *H. maydis* were brought out in the description. Stout was of the opinion that the absence of a perithecial stage and caulicolous habit of the fungus justified specific separation.

Although the virulence of the fungus was not verified, Stout felt that *Helminthosporium zeicola* was pathogenic. Examination of an herbarium specimen of *H. zeicola* was of little value for comparison with the Corn Belt species of *Helminthosporium*. The old cornstalk, while showing some breakdown of tissues at a lower node, did not present any distinct set of symptoms

comparable to that found on host material infected by the species of *Helminthosporium* under consideration. Typical spores of the genus *Helminthosporium* were found associated with the nodal tissues of the specimen, but these were so few in number and in such a collapsed condition that any comparisons could not be considered reliable.

In view of the absence of any descriptions fitting the species of *Helminthosporium* under study and the differences pointed out between it and *H. maydis* and *H. zeicola,* the two species it most closely resembles, a new species, *H. carbonum* n. sp. is proposed. The specific name is descriptive

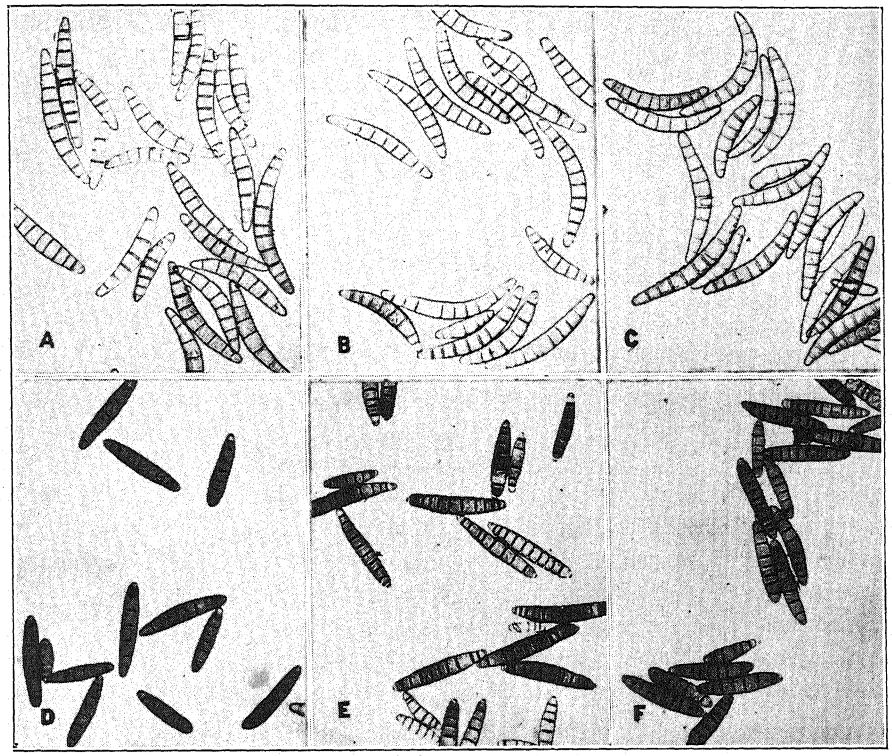


FIG. 3. A–C. Photomicrographs of spores of *Helminthosporium maydis* from three southern isolates. D–F. Photomicrographs of spores of *H. carbonum.* D and E, race I; F, race II. All photomicrographs are of spores collected from infected seedlings of K61 × Pr. Exposures and printing time identical for all illustrations. All magnifications approximately × 230.

of the distinct and characteristic charred appearance of ears of corn infected by the fungus.

Helminthosporium **carbonum** sp. nov.

Conidiis elongato-ellipsoideis, plerumque rectis vel rarissime leniter curvatis, olivaceo-brunneis, 25–100 × 7–18 μ, plus minusve circa 62.6 × 13.2 μ, 2–12 septatis, hilo obscuro; conidiophoris solitariis vel in gregibus e stomatibus erumpentibus, 90–230 × 5–7 μ obscuro olivaceo-brunneis.

Conidia 25–100 × 7–18 μ (means = 62.6 × 13.2 μ) widest in the center and tapering slightly toward rounded ends, straight or slightly curved, dark olivaceous brown, 2–12 septate (mean = 7) with hilum rather inconspicuous, and germinating by two polar germ

tubes. Conidiophores arising singly or in small groups from the stomata, 90–230 × 5–7 μ, dark olivaceous-brown, bearing one to several conidia.

Causing spots on aerial parts of *Zea mays* L. in central United States.

Type specimens deposited in the Arthur Herbarium, Purdue University, Lafayette, Indiana, and in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

The species is divided into two morphologically indistinguishable races, separable on the basis of symptoms produced and specialization in parasitism of one of these races toward certain inbred lines of corn.

Distribution: On aerial parts of *Zea mays* L. race I found throughout central Corn Belt from Iowa to Ohio and from the Ohio River to northern Indiana. Distribution dependent upon occurrence of susceptible inbred lines of corn. Race II collected from Iowa to Ohio and from Tennessee to southern Wisconsin.

Symptoms: Race I: Lesions small, pale-green or yellowish in early infections. Fully developed lesions show zonate pattern, dry light brown centers, light to purplish-brown margins. Lesions range in size from hardly visible spots to those 5 × 20 mm. Fruiting of fungus abundant on leaf sheaths and husks under humid conditions. Ears infected through tips, shanks, or directly through husks. Diseased ears covered with black mycelium imparting a charred appearance.

Race II: Lesions in early stages not unlike those caused by race I. Fully developed lesions are elongated, irregular and up to 3 × 20 mm. in size; chocolate-brown in color, zonate pattern not as distinct as in lesions caused by race I. Race II not highly specialized in parasitism toward inbred lines of corn. Corn ears more frequently attacked by this race than leaves. Symptoms on ears no different than those caused by race I.

DISCUSSION

When *Helminthosporium carbonum* was first studied, it was identified as *H. maydis* because of its similarity to the latter in morphology and symptoms produced on the host. Further studies, in which viable cultures of *H. maydis* have been compared with those of *H. carbonum*, have brought out differences between them which are believed to justify specific separation. The ranges of spore length and spore width, which are common criteria employed in descriptions, are not indicative of morphological differences between the two species. The mean values, particularly of spore length, bring out striking differences between the two fungi. Other differences such as color and curvature of the spores, characters that are not amenable to easy comparison with descriptions in the literature, have been brought out on observation of viable material of these two species.

Original isolations of *Helminthosporium carbonum* race I were made in 1938 from the dent corn inbred line Pr. Since that time two additional inbred lines K61 and K44 have been found to be susceptible. Both of these lines arose from a common stock, the open-pollinated variety Pride of Saline. Single crosses between any two of the three known suscepts are readily infected with isolates obtained from the inbred lines, indicating that the single recessive gene governing susceptibility is identical in all three lines. The dominant allelomorph has been found present in all other lines tested. It appears very probable that with continued development of new inbred lines other susceptible lines will be found. Susceptibility may well be overlooked if the material is grown in an environment unfavorable for the development of the disease. This appears to be the situation with the inbred line Pr. This line originated in Iowa where the disease did not occur, and only recently has the organism been recovered from specimens collected in that State. Obviously, if conditions there had favored the disease, the inbred line

would have been eliminated by either artificial or natural selection. After the line was brought to Indiana, in an environment as optimum for the disease as has occurred since 1938, susceptibility became apparent.

Helminthosporium carbonum race II was first observed in 1939 attacking the leaves of a proprietary inbred line. Since that time no further occurrence of leaf infection has been found in the field. Each year since that time a number of inbred lines have shown ear infections. The inbred line 187–2 appears to be particularly susceptible to ear infection. In general this race is not nearly so virulent nor so highly specialized in parasitism as race I.

During the five years that *Helminthosporium carbonum* has been under observation no indication of an ascigerous stage has been found. Diseased host material held under various environments in the laboratory as well as material subjected to the natural vicissitudes of field conditions have failed to produce a perithecial form that could be associated with the parasite under study. Likewise, a search for hosts of the fungus other than corn, has, to date, yielded negative results.

Original specimens of *Helminthosporium maydis* supplied by Mr. George Y. Young and collected in Southern States during the summer of 1942 were placed in moist chambers in an attempt to initiate the development of the ascigerous stage, *Cochliobolus heterostrophus*. A few perithecia were produced on some of the diseased specimens, but these did not reach full maturity, although held for fifty days in a humid atmosphere. The stage of development reached, however, was sufficient to make identification reasonably certain. The asci containing long ascospores in typical helicoid arrangement were clearly in evidence.

SUMMARY

Further studies are reported on a species of *Helminthosporium* attacking corn. This species, originally identified as *H. maydis,* is shown to be morphologically different from the latter. The differences in morphology brought out on comparison of viable cultures of both species, is based primarily on mean length, curvature, and color of the conidia. Differences between the species in symptoms on seedlings and specialization in parasitism are point out.

The binomial *Helminthosporium carbonum* Ullstrup is proposed; the specific term referring to the charred symptoms characteristic of corn ears infected by this fungus.

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A SEEDLING BLIGHT OF CASTOR BEAN, RICINUS COMMUNIS

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Castor beans of the Conner variety planted in crocks of quartz sand in the greenhouses of the Plant Industry Station, Beltsville, Maryland, in the spring of 1942, showed a high percentage of disease at germination. The growing points of many of the diseased seedlings rotted before emergence,

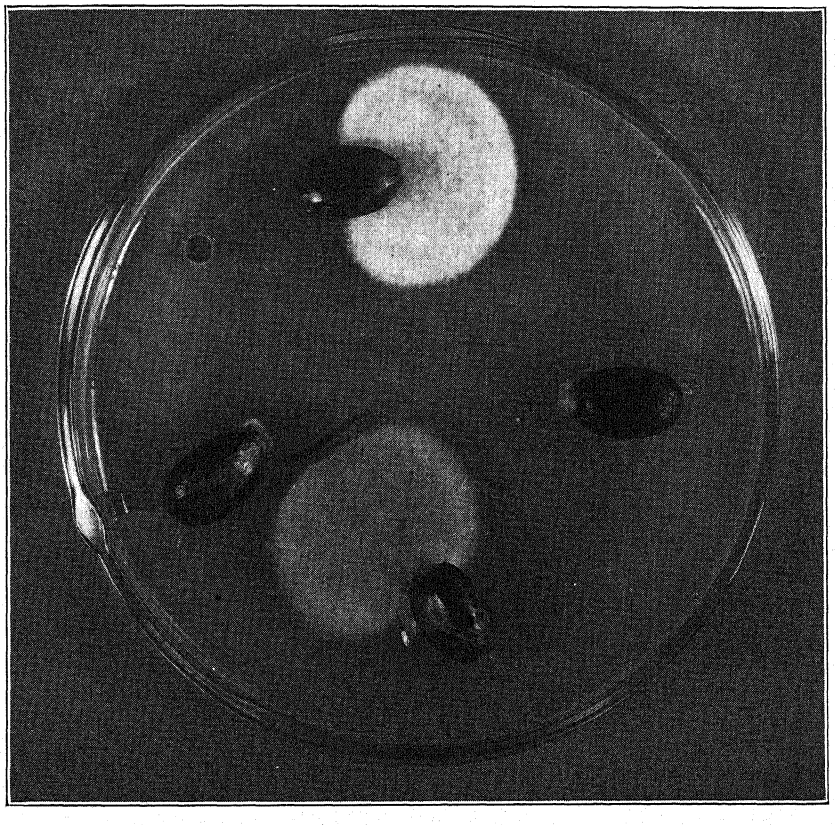


FIG. 1. Growth of *Alternaria* from castor beans of the Conner variety from Urbana, Ill., 5 days after planting surface-sterilized seeds on agar. Natural size.

or the seedlings were killed back soon thereafter. Frequently the hypocotyl rotted off just below the cotyledonary leaves. Other plants, although not killed back, suffered severe injury to the cotyledonary leaves. No spotting of the true leaves was observed, possibly because the humidity in the greenhouse did not favor such infection. Isolations from infected cotyledonary leaves and young shoots consistently yielded an *Alternaria*.

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Since the seeds had been planted in clean sand, it seemed likely that the disease was seed-borne. Accordingly, isolations were made from surface-sterilized seed from the same source (seed of the Conner variety produced by the Department of Agronomy, University of Illinois, Urbana) and from other varieties produced there and at the Plant Industry Station, Beltsville, Maryland, as well as one variety from an unknown source (Table 1). The majority of the cultures were obtained from the caruncle (Fig. 1). In a few instances *Alternaria* also was found when the seed coat was broken open and the endosperm and embryo were removed aseptically. Microscopic examination of seeds revealed the presence of mycelium throughout the caruncles but not elsewhere.

Nine lots of seed were tested for infection by culturing and by planting in sterile white sand in the greenhouse. Seed from 5 of the 9 lots yielded

TABLE 1.—*Isolation of Alternaria from castor bean seed in comparison with the occurrence of seedling necrosis in comparable lots of seeds. This seed was produced in 1941*

Variety	Seed source	Isolation from seeds		Seedling necrosis		
		No. of seeds	No. of seeds yielding *Alternaria*	No. of seeds planted	No. of seedlings	No. of seedlings with necrosis
Doughty No. 11	Urbana	25	0	12	12	0
U. S. No. 4	"	15	0	.....	.....	.....
U. S. No. 4	Beltsville	25	0	12	11	0
Conner	"	25	0	12	12	0
Conner	Urbana	95	27	12	10	10
Kentucky No. 38	"	10	4	12	9	3
U. S. No. 7	"	10	3	12	12	0
Kansas Common	"	10	5	12	12	4
Conner Improved	Unknown	20	7	12	10	0

Alternaria on culturing. Seedlings grown from 3 of these 5 lots exhibited necrosis typical of injury by this species. On the other hand, seedlings grown from the 4 lots of seed that did not yield *Alternaria* in culture, did not exhibit Alternaria necrosis (Table 1).

Seeds of the Conner variety produced both at Beltsville and at Urbana were planted in sterile quartz sand, sterile bank sand, sterile gravel, sterile soil, and unsterile soil to determine whether the type of medium in which the seedlings were grown had any pronounced effect on the severity of the disease in the seedlings (Table 2). The disease appeared in a higher proportion of the seedlings grown in quartz sand and in gravel.

In order to determine the effect of seed maturity on seed infection and whether seed infection could occur through inoculated capsules, greenhouse-produced seed of the Conner variety in 3 different stages of maturity, as well as mature Beltsville, field-produced, seed were used for inoculation tests. The stages of maturity were: (a) capsules very green and immature

TABLE 2.—*Comparative performance of Alternaria-infected (Urbana) and Alternaria-free (Beltsville) castor bean seed of the Conner variety when grown in soil, sand, and gravel*

Medium	Seed source	No. seed planted	No. seedlings	No. seedlings with necrosis
Sterile quartz sand	Beltsville	12	11	0
	Urbana	12	12	9
Sterile bank sand	Beltsville	12	11	0
	Urbana	12	12	2
Sterile gravel	Beltsville	12	10	0
	Urbana	12	8	8
Sterile soil	Beltsville	12	12	0
	Urbana	12	11	1
Unsterile soil	Beltsville	12	11	0
	Urbana	12	12	2
Totals	Beltsville	60	55	0
	Urbana	60	55	22

with the spines still pink and the seed and caruncles very succulent and easily injured; (b) capsules nearly mature, just beginning to turn brown, and a few of them beginning to split; (c) capsules mature, brown, and beginning to crack open, with spines very stiff and seed coats and caruncles very hard; (d) mature field-produced, cut September 23, 1942, after a period of heavy rain and dried in a warm greenhouse until December 4, 1942. One-half of each of the lots was shelled and the other half was left in the capsules. The various lots were dipped in a suspension of spores and mycelium of one of the Alternaria isolates and then placed in moist chambers. After 3 days in the moist chambers mycelial growth was abundant on the seeds and capsules, and the lids were removed and the seeds allowed to dry. Five days later the seeds that had been inoculated in the capsules were shelled out, washed in tap water, surface-sterilized, and planted on agar plates. Infection (Table 3) was present in seeds inoculated at all 4 stages of maturity. The appearance

TABLE 3.—*Results from inoculating seeds of the Conner variety of castor bean at various stages of maturity, by dipping the whole capsules or the shelled seeds in a suspension of spores and mycelium of Alternaria*

Where produced	State of maturity	Treatment	Condition when inoculated	No. seeds	No. seeds with *Alternaria*
Greenhouse	(a) Green	Inoculated	Shelled	26	5
			In capsules	28	10
	(b) Partly mature	Not inoculated	Shelled	46	0
			In capsules	52	0
		Inoculated	Shelled	45	5
			In capsules	48	18
	(c) Mature	Inoculated	Shelled	50	10
			In capsules	48	2
Field	(d) Mature	Not inoculated	Shelled	27	1
			In capsules	41	1
		Inoculated	Shelled	27	0
			In capsules	48	5

of some *Alternaria* in the noninoculated field-produced seed suggests slight earlier infection of these seeds. Nearly all of the growth of *Alternaria* from the seeds was from the caruncles. Occasionally there was some growth from other parts of the seeds, particularly from injured seeds, which were common in the very young, succulent material. Mycelium was present throughout the caruncles of all of the young, succulent seeds and was abundant in the nucellus. The endosperm of these young seeds had shriveled so much that the seeds were very light weight. Whether this was due to infection by the *Alternaria* or to the removal of the spikes from the plants before maturity was not determined. In the more mature seeds the mycelium was not extensive in the caruncles, being confined largely to the outer cells. A



FIG. 2. Castor bean seedlings of the Conner variety inoculated with a spore and mycelial suspension of *Alternaria compacta* at the time of emergence (right). Uninoculated seedlings (left).

Fusarium was present in a large percentage of the caruncles of the field-produced seed.

The pathogenicity of the *Alternaria* isolated from castor-bean seeds was further established by pouring a suspension of spores and mycelium over seedlings just beginning to break through the surface of sterile quartz sand. Four of 12 seedlings so inoculated were dead 14 days after inoculation; 7 others exhibited many mild to severe necrotic lesions (Fig. 2). Infection also occurred when plants 3 weeks old were sprayed with a spore and mycelial suspension and placed in a moist chamber for 4 days. Large areas of the inoculated leaves became water-soaked and flaccid (Fig. 3) and all were covered with numerous slightly zonate, dark-brown necrotic lesions approximately one-fourth inch in diameter (Fig. 4). Many of the leaves

were shed, but no stem lesions were observed. Control plants sprayed with water only and placed in the moist chamber at the same time remained healthy. Similar but less numerous and smaller lesions were observed on more mature plants inoculated at the same time.

Macrosporium leaf spots on *Ricinus communis* L. have been reported from Korea and Japan (10), from southern Russia (6, 8), and from Italy (1, 7). Tropova (8) observed losses up to 20 per cent in *R. communis* plantings due to *Macrosporium cavarae* in the Don region of Russia in 1926. Reports of leaf spots caused by *Alternaria* in Texas, Florida, and Louisiana have appeared in the Plant Disease Reporter (9), and *Alternaria brassicae*

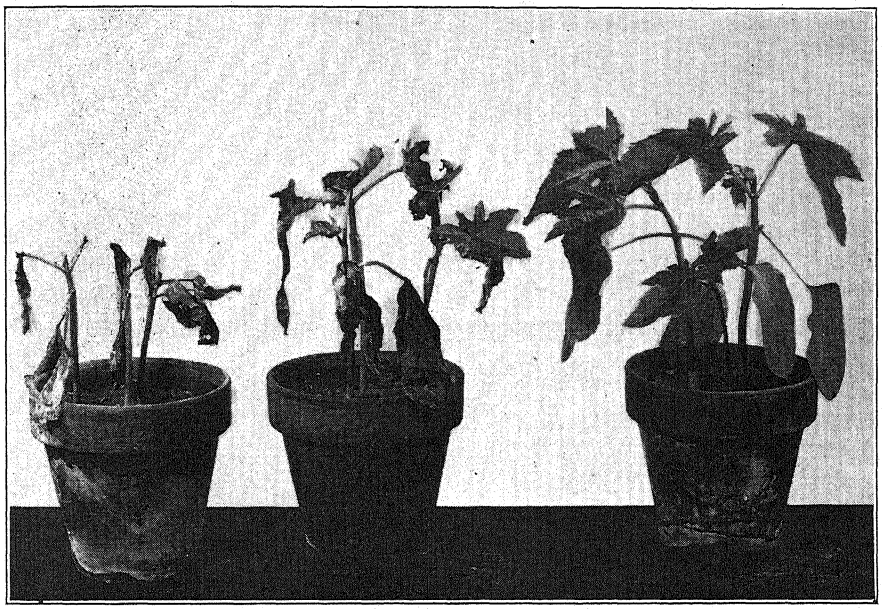


FIG. 3. Foliage of 21-day-old castor bean plants 4 days after inoculation with a spore and mycelial suspension of *Alternaria*. Healthy plants on the right.

is listed as causing a leaf spot on old plants of *Ricinus* in New York. *Alternaria* species also have been reported as causing leaf spots on *Ricinus* in Brazil (2) and in India (3). Baldacci (1) isolated *M. cavarae* and an undetermined *Fusarium* from the apices of *R. communis* seeds. He believed that the former causes a leaf spotting and attacks the apices of seeds (presumably the caruncles), whereas the *Fusarium* was thought to produce lesions on the stems, inflorescences, capsules, and seed apices.

Three species of *Macrosporium* have been described on *Ricinus communis*. Yoshii (10) described a *Macrosporium* pathogenic on castor bean leaves, which he named *M. ricini*. The spores are 42 to 78 μ long by 8.7 to 19.5 μ wide with long (30–100 μ), slender beaks, whereas the spores of the writer's organism have no elongate beaks and are smaller (14.0–38.5 μ × 7.0–19.5 μ with a mean of 21.4 μ × 10.8 μ). Parisi (7) described *M. cavarae*, which

produces round, brownish, slightly zonate leaf spots. She reports that the cotyledons and first leaves of seedlings were attacked with considerable virulence but she did not report pre- or post-emergence killing of the seedlings such as the writer has observed. The spores are described as being clavate, 34–40 μ × 10–13 μ, and having a "pedicel." Ravenel collected a fungus that was fruiting on mature stems of castor beans at Houston, Texas, in 1869, and was later described by Cooke (4) as *M. compactum*. He states

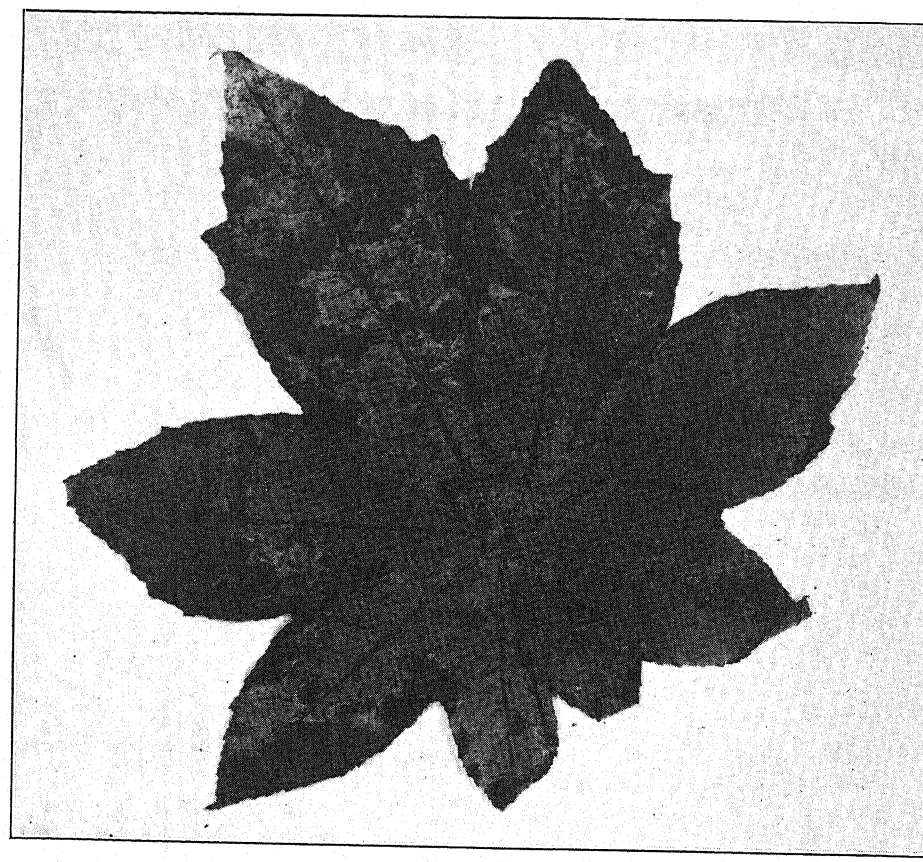


FIG. 4. Young castor bean leaf 4 days after inoculation with *Alternaria compacta*. × ⅔.

that the spores are obtuse and measure 20–30 μ × 12–14 μ. However, measurements of 12 spores from herbarium type material show a range of 15.8–38.5 μ × 7.0–17.5 μ. This is in good agreement with measurements of spores from the writer's isolates. Cooke made no mention of a leaf spot in his brief description. On the other hand, the writer has not observed *Alternaria* fruiting on the stems of his material; but, because of the similarity in the measurements and the appearance of the spores, the writer's organism is considered to be the same as the one described by Cooke as *M. compactum*. Because of the spore size and shape, *M. compactum* falls

into the *Alternaria tenuis* group of Elliott (5) and becomes **A. compacta** (Cooke) n. comb. Herbarium material of *M. cavarae* was not available for study, but it is thought to be also synonymous with *A. compacta,* since Parisi's description of the symptoms on the foliage and seedlings is very similar to the writer's observations on the disease described herein. The spore measurements she presents show *M. cavarae* as having slightly longer spores (34–40 μ), but this is not thought to be sufficient to warrant separation from *A. compacta.*

SUMMARY

A seed-borne disease of castor beans caused by a species of *Alternaria* is described. It causes pre- and post-emergence damping-off and a seedling and foliage blight, and readily infects immature seeds.

Three species of *Macrosporium* previously described on *Ricinus communis* are discussed and their descriptions compared with the writer's isolates. The writer's isolate is believed to be distinct from *Macrosporium ricini* Yoshii but identical with *M. compactum* Cooke and *M. cavarae* Parisi, which are here given the new designation **Alternaria compacta** (Cooke) n. comb.

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RELATION OF SPORE DIMENSIONS TO THEIR RATE OF FALL

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The rate at which fungus spores fall through still air constitutes an important primary element in the understanding of the possibilities of their dissemination by the wind, both locally and over long distances. Long-distance travel of spores must be taken into special account in plant-quarantine activities, because successful intercontinental transport of spores via air drifts and prevailing winds might in some cases nullify attempts to exclude foreign diseases by the customary port-inspection methods. In the study here presented an attempt was made to establish from available information on the rate of spore fall in still air a relation to spore dimensions that will permit the probable rate of fall of any spore to be determined with a fair approximation to accuracy.

Any constant relation between spore dimensions and rate of fall is likely to be reached through the spore surface, since air friction, and consequently retardation, is largely dependent on the superficial area of the falling spore. Spore surface is obtained from the formula πd^2, which becomes $\pi(l \times w)$ for oval or cylindrical spore types by substitution of the geometric mean diameter, $\sqrt{l \times w}$, for d. Since the value π is a constant, any correlation between rate of fall and spore surface must be sought through the variable $(l \times w)$ or its equivalent d^2.

The actual rates of fall of the spores of some 20 fungi have been determined experimentally,[2, 3, 4] and as the spore dimensions of these are also available the desired correlation can be attempted. Table 1 gives the determined rates of fall for these 20 spore types together with spore dimensions and other derived values needed for correlation. If the $(l \times w)$ values (microns) for these spores, and their rates of fall (mm. per sec.) are plotted independently on the same graph, each against spore diameters (microns), there are obtained two curve series of apparently similar type but not coincident. To bring the $(l \times w)$ values into a common position on the graph with the rate-of-fall series it is necessary to introduce a suitable constant. This constant is readily obtained by finding the average of the values $\frac{l \times w}{\text{rate of fall}}$ as determined for each spore. For reasons that will appear later the spore last listed in table 1, *Helminthosporium sativum*, was omitted from the calculation; the average for the remaining 19 (Table 1) was found to be very close to 40 (39.67).

In the graph in figure 1 the rate of fall for each of these 19 spores, expressed in millimeters per second, has been plotted against spore diameters

[1] United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, U. S. Dept. Agr., Washington, D. C.

[2] Christensen, J. J., in Aerobiology, A.A.A.S., Pub. No. 17, 1942; p. 81.

[3] Buller, A. H. R., Researches on Fungi, 1905.

[4] McCubbin, W. A., Phytopath. 8: 35–36, 1918.

in microns. There has also been included a curve representing an arbitrary series of the values $\frac{l \times w}{40}$ or their equivalents, $\frac{d^2}{40}$, likewise plotted against spore diameters, the perpendicular scale thus serving to represent millimeters per second for the rate-of-fall series and microns for the derived curve. It will be observed that there is a reasonably good coincidence between this curve and the points representing the determined rate-of-fall values. Insofar, therefore, as this coincidence may be relied on to represent

TABLE 1.—*Spore dimensions, observed rates of fall, and values derived from these, used in preparing the graph (fig. 1)*

Fungus	Spore dimensions (microns) (l)	(w)	Product of spore dimensions ($l \times w$)	Square root of product $\sqrt{l \times w}$	Observed rate of fall (mm. per sec.) r	Ratio $(l \times w) \div r$
Collybia dryophila	5.44	3.23	17.57	4.192	0.49	35.86
Pluteus cervinus	5.95	4.57	27.19	5.214	0.67	40.58
Paxillus involutus	7.48	4.88	36.50	6.042	1.10	33.18
Psalliota campestris (field)	7.26	5.35	38.84	6.232	1.06	36.64
Psalliota campestris (bed)	9.7	5.80	56.26	7.500	1.61	34.94
Marasmius oreades	9.5	5.6	53.20	7.300	1.34	39.70
Boletus badius	12.8	4.29	54.91	7.410	1.09	50.38
Amanita rubescens	9.38	6.53	61.25	7.826	1.54	39.77
Galera tenera	10.47	6.06	63.44	7.965	2.13	29.79
Russula emetica	8.82	7.5	66.15	8.133	1.64	40.33
Polyporus squamosus	14.6	5.13	74.89	8.654	1.03	72.72
Ustilago zeae	10.0	9.0	90.00	9.487	3.50	25.71
Coprinus comatus	12.55	7.48	93.87	9.688	3.96	23.70
Amanitopsis vaginata	10.87	10.87	118.15	10.870	2.95	40.05
Coprinus plicatilis	12.9	7.9	101.91	10.092	4.29	23.75
Alternaria sp.	20.0	10.0	200.00	14.142	3.00	66.67
Cronartium ribicola	22.0	19.0	418.00	20.445	10.00	41.80
Puccinia graminis tritici	28.0	17.0	476.00	21.817	12.00	39.67
Puccinia triticina	25.0	20.0	500.00	22.361	13.00	38.46
Helminthosporium sativum	75.0	20.0	1500.00	38.730	20.00	75.00

reality, a relation is established between simple spore dimensions and observed rates of fall, which may be used to predict the probable rate of fall of any spore in this type group, which includes spherical and oval spores as well as those of cylindrical shape with hemispherical ends. The formula expressing this relation is: $\frac{l \times w}{40} = r$, where l is spore length in microns, w the breadth in microns, and r the rate of fall in millimeters per second.

Spores of fusiform type cannot be incorporated directly into this simple formula series. In computing the surfaces of the two cones set base to base, which make up the simplest type of fusiform spore, one has to employ the slant side of the cone, a value that does not vary directly with the spore dimensions l and w. Moreover, some types of fusiform spores are not con-

structed on the double-cone pattern, but may be built on the model of a cylinder set between two cones, the length of the intercalated cylinder usually having no fixed relation to the total spore length, l.

The surfaces of both types of fusiform spores can be readily calculated, however, and if it is assumed that falling rate here has the same relation to spore surface as in the case of spherical, oval, or cylindrical spores, then one can easily obtain a formula to cover each case.

For double-cone spores this formula is $\frac{w \times s}{40} = r$, where w is spore width, s the slant side of one cone element, and r the rate of fall in millimeters per

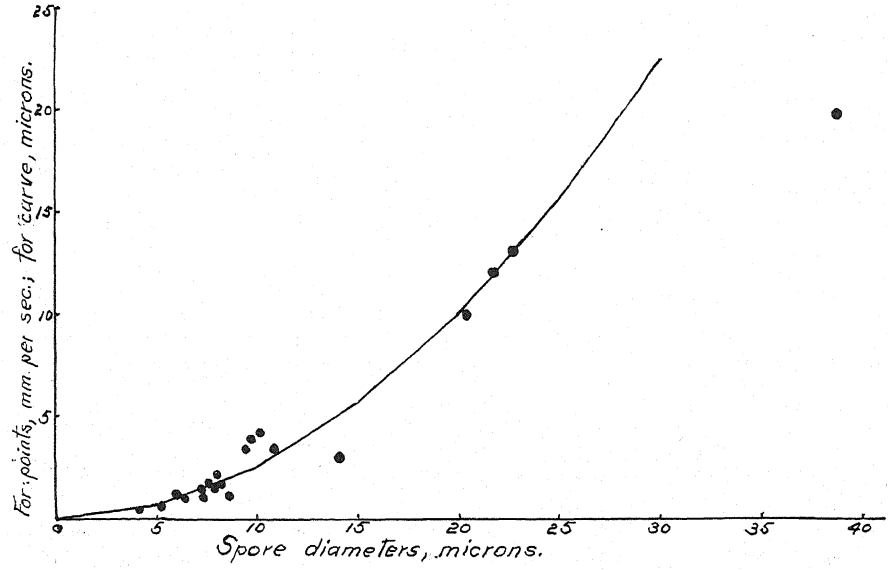


FIG. 1. Observed rates of fall (mm. per sec.) for the spores listed in table 1 are plotted against spore diameters (microns) to give the point series. For the curve, spore diameters, d, of a hypothetical series of spherical spores are plotted against $\frac{d^2}{40}$ (microns) to indicate that the value of $\frac{d^2}{40}$ in microns is closely comparable with the rate of fall in millimeters per second.

second. In terms of the ordinary spore dimensions, l and w, this becomes $\frac{w \times \sqrt{l^2 + w^2}}{80} = r$.

For most ordinary needs where the mathematical accuracy of this formula is not required, the simple formula $\frac{l \times w}{80} = r$ will give an approximately correct falling rate. It merely substitutes the axial cone length $\frac{l}{2}$ for the slant side s, on the assumption that in slender spores these values are so nearly identical that the error introduced by the substitution will be very small.

A more accurate empirical formula substitutes for s the axial cone length plus a variable fraction expressed in terms of l and w, which thus gives a value approximately equal to s. This formula is:

$$\frac{w\left(l+\frac{w^2}{2l}\right)}{80}=r$$

This formula is surprisingly accurate for spores in which the ratio of l to w is above 2:1.

For fusiform spores consisting of two cones and an intercalated cylinder of the same axial length as each cone element, that is, cylinder length $=\frac{l}{3}$, the mathematical formula for falling rate is:

$$\frac{w\left(\sqrt{\left(\frac{l}{3}\right)^2+\left(\frac{w}{2}\right)^2}+\frac{l}{3}\right)}{40}=r$$

For the same spore type the empirical formula is:

$$\frac{w\left(\frac{2l}{3}+\frac{3w^2}{8l}\right)}{40}=r$$

And the simple approximate formula is: $\frac{l\times w}{60}=r$

For the general fusiform type of spore it is necessary to introduce another symbol, x = axial length of one cone, to obtain this formula:

$$\frac{w\left(\sqrt{x^2+\left(\frac{w}{2}\right)^2}+l-2x\right)}{40}=r$$

The corresponding empirical formula is:

$$\frac{w\left(l-x+\frac{w^2}{8x}\right)}{40}=r$$

and the simple approximate formula $\frac{w(l-x)}{40}=r$

It will now be apparent that the spores of *Helminthosporium sativum* were omitted from the initial calculation because they fall into another spore group from the present point of view, belonging somewhere in the fusiform types, which have somewhat different relations of surface to the dimensions l and w. If one considers that these spores come fairly close to the type represented by two cones and a central cylinder of about cone length, the calculated falling time would be 26 mm. per second, according to both the mathematical and empirical formulae and 25 mm. per second for the approximate formula. The observed falling time was 20 mm. per second.

In presenting this study of the relation between ordinary spore dimensions and their rate of fall, it is emphasized that no high degree of accuracy should be expected from the formulae given; they are derived from the average values of a scanty series of determinations; hence, the probability

of error is high. It is hoped that when additional spore-fall determinations are available a recheck of these relations will bring something closer to reality. Also these formulae leave out of consideration the changing relation of spore surface to spore volume among the members of a size series of spores. With size increase goes an increased volume per unit of spore surface, resulting in greater gravitational pull earthward in proportion to air resistance. Nor is it wise to overvalue the rate of spore fall that can thus be predicted. Other factors outside the scope of this prediction method, such as spore density, peculiarities of shape, and irregularities in surface configuration, introduce such great variations into the rate of fall that prediction may become in some cases uncertain or of diminished worth. One may be confident, however, that with all these shortcomings the rate of spore fall will always serve as a sound and orderly point of departure for estimating the possibilities of long-distance spread of pathogens in air currents. When these extremely slow rates of fall are contrasted with the relatively imposing velocities attained by wind movements or even by slight convection currents, it is apparent that the feeble tendency of fungus spores to drift earthward under the pull of gravity can have very little effect in preventing their dissemination in favorable air currents for very long distances over the earth's surface.

In closing it may be mentioned that this spore-fall problem should constitute an excellent student exercise, serving in simple fashion to present a concrete picture of spore movement in still air, and particularly to direct attention to the altered physical relations that obtain in the size plane of fungus spores.

A TECHNIQUE FOR TESTING RESISTANCE OF COTTON SEEDLINGS TO THE ANGULAR LEAF SPOT BACTERIUM

RICHARD WEINDLING[1]

(Accepted for publication August 30, 1943)

In the course of pathogenicity tests with isolates of *Phytomonas malvacearum* (E.F.S.) Bergey, the cause of angular leaf spot of cotton, indications were obtained that seedlings might behave in a manner similar to mature plants with respect to varietal resistance against the organism. The technique presented here was developed to test this hypothesis. In preliminary experiments inoculation of seed prior to planting proved far superior to seedling inoculation in furnishing varietal differentiation with respect to disease resistance. Similar results have been reported for bacterial blights of beans.[2,3]

It would obviously be desirable to choose for such a study a series of varieties of known resistance. Unfortunately no critical field data were available, except the well-established evidence that varieties of Egyptian cotton, *Gossypium barbadense* L., are much more susceptible than varieties of upland cotton, *G. hirsutum* L. The materials used here were selected on the basis of two field trials conducted at Clemson, South Carolina, in 1942. Plants of 20 varieties and lines were inoculated by spraying with bacterial suspensions according to the method of Knight.[4] The following 5 varieties[5] are representatives of the range of resistance exhibited by the 20 varieties in the field: S × P Egyptian (extremely susceptible), Shafter Acala (highly susceptible), Rogers' Acala (moderately susceptible), Stoneville 4–5 (tolerant), and Stoneville 4–8 (resistant). Seed lots of these 5 varieties were used with the method to be described.

PRINCIPAL FEATURES OF THE METHOD

1. Reaction is tested separately to "external" and "internal" inoculation of seed, by immersing them in bacterial suspensions for short and for long periods.

2. Seedlings are grown for three weeks under conditions favorable for infection of cotyledons, i.e., abundant water supply and high temperature.

[1] Agent (Associate Pathologist), Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the South Carolina Agricultural Experiment Station.

[2] Zaumeyer, W. J. The bacterial blight of beans caused by *Bacterium phaseoli*. U. S. D. A. Tech. Bull. 186. 1930.

[3] Stapp, C. Verfahren zur Prüfung von Bohnen (*Phaseolus vulgaris*) auf Resistenz gegen *Pseudomonas medicaginis* var. *phaseolicola* Burkh., den Erreger der Fettfleckenkrankheit. Angew. Bot. 15: 241–252. 1933.

[4] Knight, R. L., and T. W. Clouston. The genetics of black-arm resistance. Jour. Genetics 38: 133–159. 1938.

[5] In order to simplify the discussion, varieties and lines are designated here as varieties. For kindly supplying the seed lots thanks are due to C. J. King, G. J. Harrison, C. H. Rogers, and D. M. Simpson. Stoneville 4–5 and 4–8 are lines obtained from Mr. Simpson. The delinted seeds of the lots were free of seed-borne pathogens.

3. Relative disease rating is based on severity of lesions as well as on rapidity of their development.

Procedure

A suspension of a recent isolation of *Phytomonas malvacearum* was prepared from a streak culture grown for 4 days on potato-dextrose agar in a Petri dish. The bacterial masses were suspended in 600 cc. of distilled water and filtered through 4 layers of cheesecloth. By means of dilution cultures the number of viable bacteria was estimated at 2.5 million per cc.

Inoculation of seed was carried out by immersing them in the bacterial suspension, using 25 cc. for each sample of 60 acid-delinted seed. One

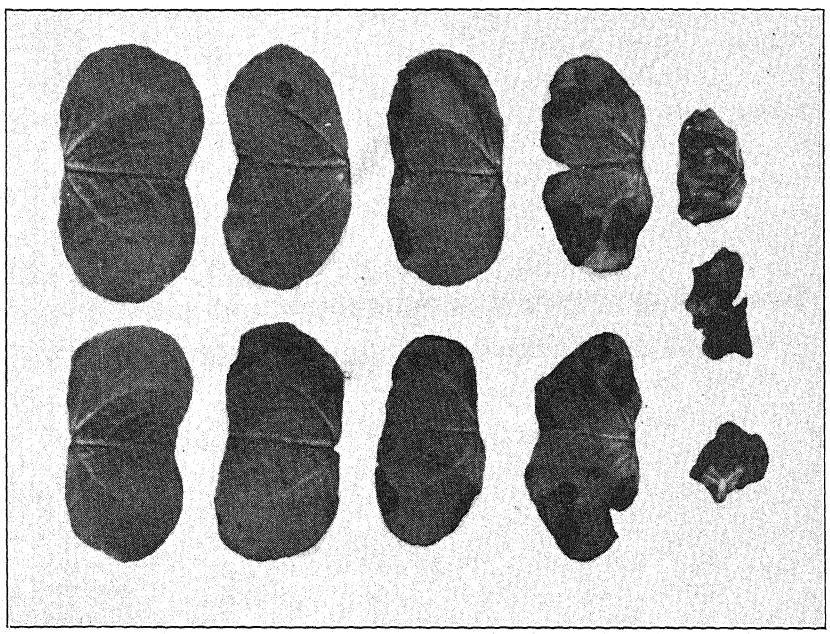


FIG. 1. Cotyledons of cotton seedlings infected with *Phytomonas malvacearum*. Degrees of severity from left to right: control, slight, severe, very severe, practically destroyed. Last row at right is S × P Egyptian; the others are Shafter Acala. Photographed 6 days after the seeds were inoculated and planted. Natural size.

sample of each variety was left in the suspension for 5 minutes, the other for 3 hours at 24–26° C. The flasks were agitated at intervals. Finally, the suspensions were decanted and the seeds were dried on paper toweling.

The seeds were placed between layers of paper toweling that were kept moist by standing in tumblers partly filled with water. This method is a modification of techniques described by Simpson, *et al.*[6] A towel consisted of two thicknesses of paper and measured 13.5 × 34.0 cm. Twelve seeds were arranged on the moistened towel 2 cm. from one margin, leaving one-half of the towel free to be folded over them. The resulting rectangle of 13.5

[6] Simpson, D. M., *et al.* Anatomical structure of the cottonseed coat as related to problems of germination. U. S. D. A. Tech. Bull. 734. 1940.

× 17.0 cm. was placed against the inner wall of a glass tumbler of 300 cc. volume. The seeds were held by the toweling at the upper edge of the tumbler with the 2 cm. margin extending beyond the glass. The paper towel thus formed a wick during seed germination and allowed the roots to grow between the layers toward the bottom of the tumbler. A 10 × 15 cm. filing card was placed within the tumbler in order to hold the toweling appressed to the wall.

No attempt was made to provide aseptic conditions. The towels were thoroughly moistened in distilled water, the excess water being removed by pressure. At the beginning, 50 cc. of distilled water was poured into each tumbler; later, tap water was added when needed to keep the water level at ½–⅔ the height of the tumbler.

The tumblers were kept for 4 days in incubator at 27–30° C. in a saturated atmosphere. On the fourth day the seed coats were removed to prevent development of mold on the towels; when necessary the seedlings were arranged so that the cotyledons were just above the upper margin of

TABLE 1.—*Factors for calculation of disease index of table 2*

Group No.	Symptoms of cotyledons		Factor
	Severity	Day after planting	
1	Practically destroyed	6th	1.0
2	Very severe	"	0.9
3	Severe	"	0.8
4	Slight	"	0.6
5	Severe	11th	0.6
6	Slight	"	0.4
7	"	16th	0.2
8	"	21st	0.1

the towel; and the seedlings were thinned to 10 per tumbler, leaving 50 seedlings of each sample in 5 tumblers. The tumblers were then moved to trays on a greenhouse bench. Air temperatures were held above 27° C. by a heating cable with thermostatic control and below 35° C. by cheesecloth shades, which protected the tender seedlings from the mid-day sun.

On the sixth day after planting, and subsequently at 5-day intervals, seedlings with lesions were removed from the tumblers and graded according to severity of symptoms (Fig. 1 and Table 1). For the purpose of these experiments, the term lesion is used only for typical bacterial spots, dark-green in reflected light and translucent by transmitted light. Computation of the disease index for each sample of 50 seedlings is explained in tables 1 and 2. The frequent removal of lesioned seedlings helped in suppressing secondary inoculation of seedlings from adjoining diseased cotyledons. In order further to preclude such spread, precautions were taken to prevent wetting of cotyledons.

RESULTS AND DISCUSSION

It is evident from table 2 that the seedling data on relative varietal resistance to *Phytomonas malvacearum* agreed well with the field experiments.

Results of a second experiment checked those of the first one in all essential points. Further tests with other varieties and lines have given similar results. The data can thus be safely interpreted as meaning that, in general, varietal reaction of seedlings inoculated by the described method conforms with observations on varietal behavior of field plants. It would seem worthwhile, therefore, to consider the technique from the standpoint of its possibilities as an aid in breeding cotton varieties for resistance to angular leaf spot.

The advantages of the method are obvious: Saving in time and labor, ease and uniformity of inoculation, and possibility of controlling environmental factors. Details of the technique may be considerably simplified or adapted to the material under test. Planting the inoculated seed in pots

TABLE 2.—*Varietal reaction of cotton seedlings to angular leaf spot. Seed inoculation. Seedlings grown for 21 days at 27–35° C. Varieties arranged from left to right in order of increasing disease resistance in the field*

	Period of inoculation	Varieties and lines				
		S × P Egyptian	Shafter Acala	Rogers' Acala	Stoneville 4–5	Stoneville 4–8
Percentage diseased	5 minutes	100.0	92.0	48.0	64.0	18.0
	3 hours	100.0	100.0	86.0	66.0	28.0
Disease index[a]	5 minutes	71.2	42.2	10.2	21.0	5.0
	3 hours	91.0	56.4	47.0	22.2	6.0

[a] Calculation of disease index from a sample of 50 seedlings: The number of seedlings falling in each group of table 1 is multiplied by the corresponding factor. The sum of these products is doubled in order to make 100 the highest disease index possible for the 50 seedlings.

or tumblers with steamed sand was in use even before the more tedious paper-towel method. The latter was adopted because the individual seedlings could be kept under close observation. This is important in the early stages of development when seedlings of very susceptible varieties are severely injured. Comparison of such varieties as S × P Egyptian and Shafter Acala is facilitated by a disease index, such as that of table 2. The more resistant varieties may be fairly well differentiated according to percentage of seedlings affected by disease.

A method of inoculating fuzzy seed by spraying with bacterial suspension[7] was tried and gave results similar to those reported here. For the purpose of these tests acid-delinted seed has the advantage of being practically free of seed-borne pathogens.

The use of 2 periods of inoculation (5 minutes and 3 hours) has distinct value for differentiating upland cottons with respect to varietal resistance (Table 2). When delinted seed is kept in the bacterial suspension for 3 hours, the seeds swell sufficiently to allow the entrance of bacteria, corresponding to seed internally infected in the field before cotton is picked. Submerging the seed in the bacterial suspension for 5 minutes deposits bac-

[7] Unpublished method, obtained from C. J. King.

teria on the seed coat in a manner simulating the natural condition of seed carrying inoculum externally. Attempts to replace the two procedures by a single one have not been promising. An intermediate inoculation period of 30 minutes did not always produce intermediate infection. The large variations in infection obtained from this treatment are probably due to variability in swelling of seed during the period of inoculation.

The reaction of the two Stoneville lines differed from that of the other varieties. The latter became more severely infected following the 3-hour inoculations than after the 5-minute exposure, while the Stoneville lines showed little or no differences. This behavior seemed so puzzling that, in repeating the experiment, the number of replications was doubled for Rogers' Acala and Stoneville 4–5. The results were consistent with those of table 2. A possible explanation was suggested by the frequent appearance of necrotic spots on Stoneville 4–8, when seed was inoculated for 3 hours. Moreover, when seed of this line was kept in the inoculum for 24 hours before planting, typical bacterial lesions were neither more severe nor more numerous than following the shorter exposures, but more necrotic spots developed. Perhaps, the Stoneville 4 lines possess a genetic factor of resistance that involves hypersensitivity of the cotyledons, causing the affected cells to die so rapidly that the parasite has little chance to produce the typical lesions.

SUMMARY

A technique is described for testing resistance of cotton seedlings to the angular-leaf-spot bacterium. Seed was inoculated by immersing in suspensions of bacteria for short and lengthy intervals. Seedlings were grown for 3 weeks at 27–35° C. In general, varietal reaction to the disease organism in these seedling tests conformed with that of field plants. The method offers possibilities as a rapid supplementary test in breeding varieties for resistance to the disease. For this purpose the technique may be adapted to the material under test by modifications, such as the use of sand culture in place of the paper-towel technique described here.

A METHOD OF INDUCING BARK-SHELLING FOR TREATMENT OF CERTAIN TREE DISEASES

H. S. FAWCETT[1] AND L. C. COCHRAN[2]

(Accepted for publication August 25, 1943)

There are certain diseases of trees which affect primarily the outer layers of bark and which may be treated in their early stages by eliminating these diseased parts. It is desirable to develop an easily applied method of accomplishing this tissue elimination without too severe injury to the tree and without excessive cost in material and labor.

For the past 20 years or more the senior writer has discussed with his associates and others the desirability of finding a substance that could be safely applied to the bark of citrus trees and would cause the outer half, or more, of the bark to shell off, thus to imitate the standard method of scraping the bark for treatment of citrus trees affected with psorosis (California scaly bark).[3] The difficulty that arose was to find a substance that could be applied to bark of varying thicknesses and accomplish such shelling without causing severe injury where the bark was thin. It is believed that, with certain limitations and reservations, a method has been found for this treatment of citrus trees. The ideal substance sought is one that can be easily applied and will penetrate and kill rapidly only the outer bark tissue but one which would not penetrate and kill the cambium. This would allow the cambium to continue growth and generate new bark promptly. The old bark with its accompanying psorosis lesion would then dry and shell off. The advantage of this method over the standard scraping method would be the greatly reduced cost of labor and increased efficiency and ease of treating crotches and irregular surfaces.

Some experiments involving this method along with others were made as early as 1922,[4] but the first trial of the more recent series of experiments with the present method was made at the Rancho Sespe, near Fillmore, California, in 1937. Dinitro-o-cyclohexylphenol at a concentration of 2 per cent by weight dissolved in dormant-spray oil was applied with a brush to psorosis bark lesions of two Valencia orange trees. After a few months slabs of bark shelled off, exposing newly-formed bark beneath.

In May and June, 1939, a more extensive experiment[5] was carried out at the Los Alisos Ranch near El Toro, California, to compare this and other methods with the standard scraping treatment of psorosis bark lesions on

[1] Professor of plant pathology and plant pathologist in the Citrus Experiment Station, University of California.

[2] Senior plant pathologist, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture.

[3] Fawcett, H. S. Citrus diseases and their control. 656 pp. 187 figs. McGraw-Hill Book Company, New York, N. Y.

[4] In cooperation with M. B. Rounds, liquor cresolis compositus was used at Azusa, California, and caused some bark shelling.

[5] This was started in cooperation with Paul Sloop and E. T. McFadden. Assistance was given by J. M. Wallace in this and subsequent experiments.

Valencia trees. Dinitro-o-cyclohexylphenol (subsequently to be referred to as DNOCHP) at 2% dissolved in dormant-spray oil was brushed on the lesions of 10 trees, and 40 trees were given the standard scraping treatment.

TABLE 1.—*Degree of injury and of bark shelling from application of various solutions to 15-year-old Washington Navel and Valencia orange trees, 18 months after treatment*

Material	Var.	Trunk		Limbs 2 to 4″ dia.		Limbs Less than 2″	
		Injury	Shelling	Injury	Shelling	Injury	Shelling
Dormant spray oil alone	Nav.	0[b]	0[c]	0[b]	0[c]	0[b]	0[c]
	Val.	0	0	0	0	0	0
⅛% DNOCHP[a] in dormant-spray oil	Nav.	0	0	0	0	0	0
	Val.	0	0	0	0	0	0
¼% DNOCHP in dormant-spray oil	Nav.	0	1	0	0	0	0
	Val.	0	2	0	2	M	1
½% DNOCHP in dormant-spray oil	Nav.	0	1	0	1	0	0
	Val.	0	2	0	2	Sl.	2
1% DNOCHP in dormant-spray oil	Nav.	0	3	0	2	0	1
	Val.	0	3	0	3	Sev.	1
2% DNOCHP in dormant-spray oil	Nav.	0	3	0	3	M	3
	Val.	0	3	0	3	Sev.	1
1% DNOCHP + 2% glycerol mono-oleate in medium grade citrus-spray oil	Nav.	0	3	0	2	0	1
	Val.	0	3	0	3	M	2
2% DNOCHP + 2% glycerol mono-oleate in medium grade citrus-spray oil	Nav.	0	3	0	3	Sl.	3
	Val.	0	3	Sl.	3	Sev.	1
2% DNOCHP + ½% aluminum stearate + 1% glycerol mono-oleate in medium grade citrus-spray oil	Nav.	0	3	Sl.	3	Sev.	2
	Val.	Sl.	3	Sl.	3	Sev.	1
½% Aluminum stearate in medium grade ctirus spray oil	Nav.	0	3	0	3	Sl.	3
	Val.	0	3	Sl.	3	Sev.	0
½% DNOCHP in medicinal grade white mineral oil	Nav.	0	1	0	1	0	1
	Val.	0	2	0	1	0	1
1% DNOCHP in medicinal grade white mineral oil	Nav.	0	3	0	3	0	3
	Val.	0	3	0	3	0	2
2% DNOCHP in medicinal grade white mineral oil	Nav.	0	3	0	3	0	2
	Val.	0	3	0	3	0	3
2% DNOCHP in kerosene	Nav.	0	3	0	3	0	3
	Val.	Sl.	3	Sl.	3	0	1
0.8% DNOCHP + 2½% diamylphenol in kerosene	Nav.	0	3	0	3	0	2
	Val.	0	3	0	3	0	2

[a] DNOCHP = dinitro-o-cyclohexylphenol.
[b] 0 = no injury; sl., slight inj.; M, med. inj.; sev., severe inj.
[c] 0 = no bark shelling; 1, slight bark shelling; 2, med. but incomplete shelling; 3, complete or nearly complete shelling.

Although some patches of cambium were killed in the chemically treated areas, especially on the limbs where the material was applied heavily, the results were essentially the same as were obtained earlier at the Rancho Sespe.

The bark was painted not only over the lesions, but in a border of about 6 in. above and below and about 3 in. on the sides of each lesion. After a few months slabs of bark including the lesions shelled off, and the cambium, with the exception of a few areas, had produced new bark under the treated areas. Although the 2% DNOCHP in dormant-spray oil was found on some trees to cause too much injury for safe general use, the effects compared favorably, especially on the trunks, with those of the standard scraping method. Whenever the material was allowed to run down to the soil line and collect there, much injury resulted from death of the cambium.

These favorable results, when precautions were taken in application, suggested the possibility of producing the desired bark-shelling without injury to the cambium by decreasing the concentration of the DNOCHP in the oil, or by using oils of other viscosities as carrier solvents, or by using other solute materials. An experiment was set up using 19 different solutions.[6] These were applied in November, 1941, each to one healthy Valencia and one healthy Washington Navel orange tree, 15 years of age, at the California Citrus Experiment Station. The materials were applied to a strip of bark extending from the ground line up the trunk and on to limbs varying in size from about 4 inches down to $\frac{1}{2}$ inch in diameter. In April, 1942, the applications were repeated on this same set of trees on portions of bark not treated the previous November. The results of fifteen of these tests for November, 1941, are shown in table 1, 18 months after treatment. The manner of bark shelling in two of the above tests is shown in figure 1. The results of the April test were similar. Four of the materials that were used are not listed in table 1, since they caused no shelling of bark. These were 2% DNOCHP in cottonseed oil, 1% DNOCHP in boiled linseed oil, 1% DNOCHP in glycerine, and 1% DNOCHP in petroleum jelly. The first three caused neither injury nor shelling, the last caused a very slow injury without drying or shelling, even after 16 months. Table 1 indicates that under the conditions of these tests, DNOCHP in concentrations below about 1% in dormant-spray oil is not effective in inducing good shelling of the bark. In concentrations higher than 1% it tends to cause some injury.

Nine of the most promising of the original 19 materials, as well as kerosene alone, were tried at the Azusa Foothill Ranch in May, 1942, on psorosis lesions of Washington Navel and Valencia orange trees. Five of these materials were selected for a test in November, 1942.[7]

Another test of the same 19 materials and of kerosene alone was made on psorosis lesions on Valencia orange trees at Rancho Sespe in July, 1942. With one exception, results were similar to those on healthy bark at the Citrus Experiment Station, but more injury was caused by the stronger solutions; kerosene alone caused no appreciable effect if not allowed to run down into the soil. Ten of the most promising of these materials were tried in another experiment at Rancho Sespe in October, 1942. Five of these and

[6] Acknowledgment is due Dr. A. M. Boyce, who from his experience with solvents for this compound, helped to devise the formulas and had them prepared for the experiment.

[7] The senior writer was assisted by M. B. Rounds in this experiment.

five additional ones, with lower specific gravity oils as solvents, were tried in December, 1942.[8] The results of the applications of the most promising solutions, such as 1% DNOCHP in kerosene, in gasoline, or in white mineral

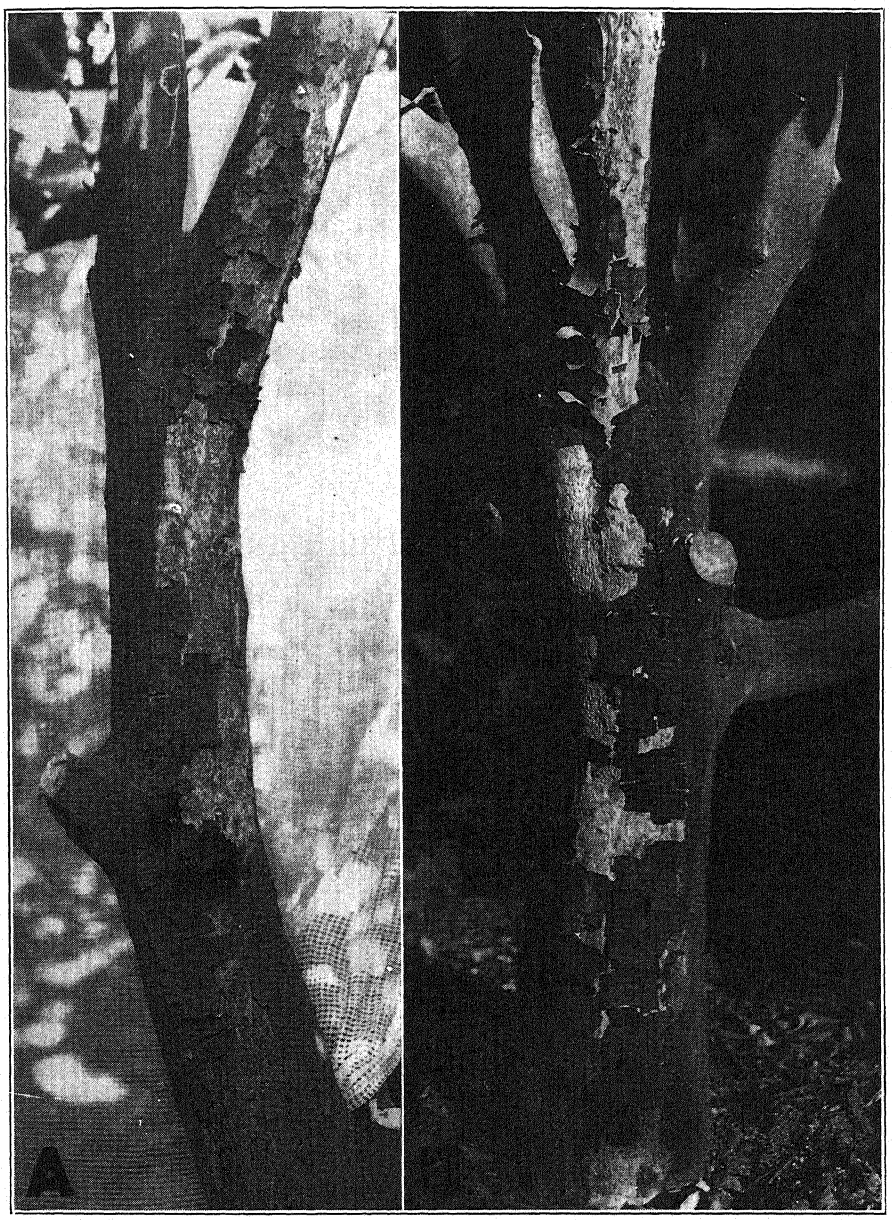


FIG. 1. A. Normal bark of navel orange treated with 2% DNOCHP in kerosene. B. Normal bark of Valencia orange treated with 2% DNOCHP in white mineral oil. New bark can be seen where the old bark scales have fallen away.

[8] The Rancho Sespe, through Howard Pressey and T. A. Lombard, gave much assistance in carrying out these experiments.

oil, to bark containing psorosis lesions showed that the cambium under the lesion is somewhat more subject to injury than that under normal bark. Some of the cambium within the lesion area is sometimes killed when none of that outside the lesion is destroyed. This takes place probably because new bark, developed in the sequence of the disease, is thinner in certain areas than normal bark. If the lesions are small, this result is not at all serious. It is also probable that use of two different strengths may be advisable, one for the trunk and larger limbs and the other for smaller limbs. In case of psorosis of citrus, however, it will not be advisable in any case to treat lesions on limbs much smaller than about 2 inches in diameter. Such limbs should be cut out instead of being treated.

SUMMARY

The results of these experiments indicate a promising chemical method for inducing bark-shelling with consequent production of new bark in psorosis lesions of orange trees. The advantages of such treatment over the bark-scraping method now in practice are obvious. This principle may have an application in the case of other bark diseases where the causal agent can thus be removed or the disease impeded by scraping or bark scarification. It is postulated that the best carrier for the toxic agent for such purpose will prove to be one that is only partially miscible with water and will, therefore, not too readily penetrate the bark and kill the cambium.

The two preparations so far tested that show the most promise in producing bark-shelling and at the same time do not seriously injure the cambium when applied with a brush to Valencia and Washington Navel orange bark, are dinitro-o-cyclohexylphenol (DNOCHP) at a concentration of approximately 1% by weight dissolved in kerosene and the same material dissolved in medicinal grade white mineral oil. A few tests indicate that ordinary gasoline without tetra-ethyl lead may also be a good carrier for the DNOCHP but in warm, dry weather, it evaporates too rapidly.

Lighter penetrants, carrying DNOCHP or other substances, are being tested.

BOTRYTIS LEAF SPOT OF VETCH[1]

J. L. Weimer

(Accepted for publication, August 4, 1943)

INTRODUCTION

In March, 1940, Mr. George E. Ritchey, Gainesville, Florida, sent the writer some Augusta vetch (*Vicia angustifolia* L.) plants having numerous red spots on leaflets, petioles, and stems. These spots differed somewhat from those caused by any disease with which the writer was familiar or found described in the literature at the time. Although Augusta vetch grows wild in many parts of the South, it is used commercially only to a very limited extent, largely in Florida. Since this disease was evidently capable of doing considerable damage to Augusta vetch and possibly was potentially destructive to other vetch species of more commercial value, an investigation of its nature and cause has been made.

SYMPTOMATOLOGY

All aboveground parts, at least of young plants, are susceptible. On the leaflets the spots tend to be circular in outline but may be angular or irregular, especially when they are restricted by the larger veins. There may be only one spot on a leaflet or spots may be so numerous that most of the tissue of the leaflet is involved. These lesions are characteristically small, often from $\frac{1}{3}$ to $\frac{3}{4}$ mm. in diameter, and seldom over 1 mm. unless formed by the coalescence of two or more adjacent spots, or their growth is diverted by contact with veins. Their color may vary, but, usually, it is a shade of dark-red approaching oxblood of Ridgway.[2] The spot may be uniform in color or it may be ox-blood with a maroon border, or sometimes the border is claret-brown, mahogany-red, or chestnut, fading towards the center to light-brown, gray, or almost white. The spots may appear on either side of the leaflet and may or may not extend entirely through it. The affected areas are not excised but the leaflets are shed and a plant may be badly defoliated. On the stems and petioles the lesions are similar to those on the leaflets except they are commonly linear in shape, the longest axis being parallel to the corresponding axis of the part attacked. Such lesions are usually from $\frac{1}{2}$ to 1 mm. wide and up to 2 to 3 mm. long, but may enlarge, becoming 1 cm. or more long. The spots may continue to enlarge and coalesce with nearby lesions, so that girdling and death may result, small petioles and tendrils most commonly being killed in this manner. Damage to the plant results

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 117, Journal Series, Georgia Agricultural Experiment Station.

[2] Ridgway, Robert. Color Standards and Color Nomenclature. 43 pp., 53 color plates. (Washington, 1912.)

from the girdling of the parts affected, but perhaps more generally from defoliation. Considerable defoliation may result when the spots are numerous, as they often are under natural conditions (Fig. 1, A).

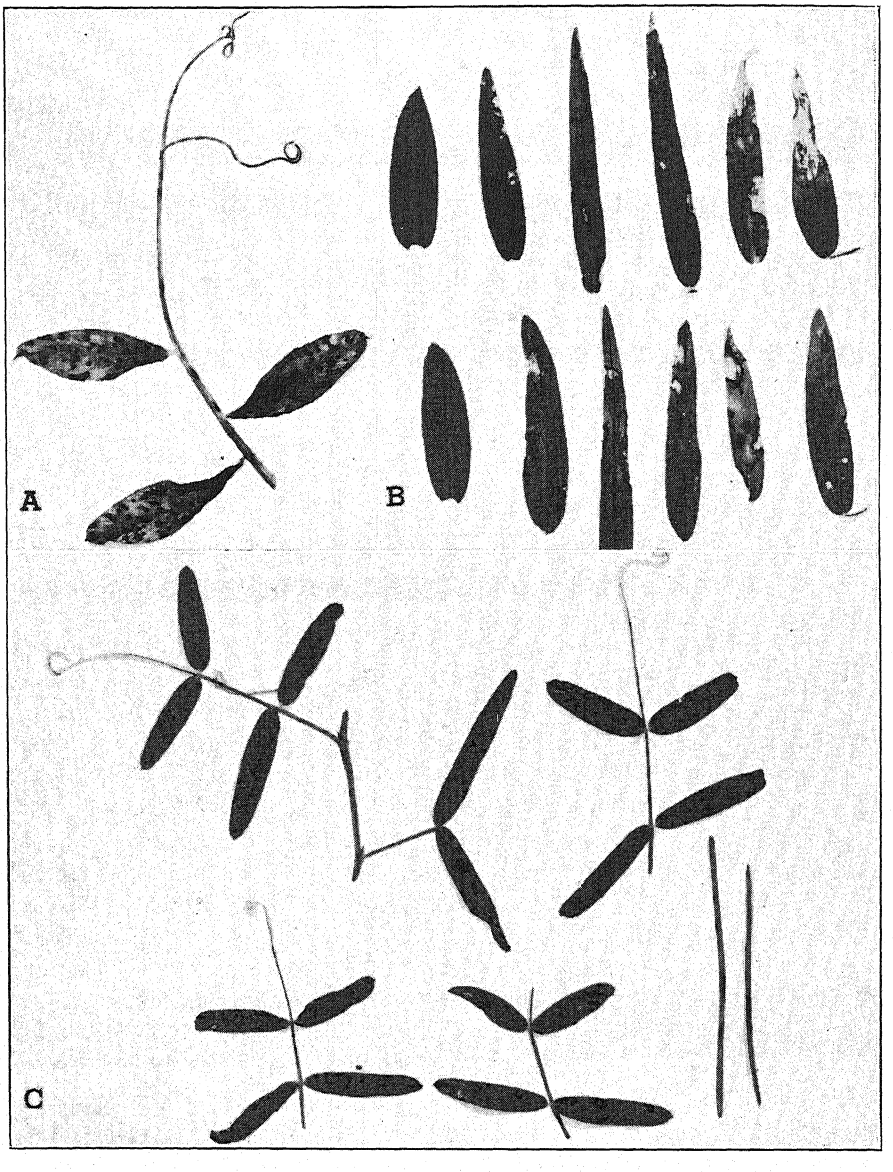


FIG. 1. *Botrytis* leaf spot of vetch. A. Naturally infected leaflets, leaf stalk, and tendril of *Vicia angustifolia* from Gainesville, Fla. ×6/7. B. Botrytis leaf spot on *V. grandiflora* resulting from inoculation. Two leaflets at the left are healthy. Upper and lower rows of leaflets show upper and lower surfaces, respectively. ×7/8. C. Botrytis lesions on leaflets, leaf stalks, stems, and tendrils of *V. sativa* (Alba) resulting from inoculation. About ×9/10.

ETIOLOGY

Pathogenicity

Isolations from the leaves received from Ritchey and from plants collected later by the writer showed the constant association of a *Botrytis* with these lesions. Inoculations demonstrated that the typical symptoms could be reproduced at will. On January 22, 1942, young plants of *Vicia angustifolia* grown in sterilized soil in the greenhouse were atomized with a suspension of spores from a young pure-line culture of the fungus growing on potato-dextrose agar and were then held in a moist chamber for 48 hours. Comparable control plants atomized with water only were placed in the moist chamber along with those inoculated. At the end of 48 hours, when the plants were removed from the moist chamber, there were numerous water-soaked, slightly sunken spots present on some of the leaflets of the inoculated plants but none on those of the controls. Four days later the spots had taken on the red color characteristic of the Botrytis disease. The fungus was recovered from several of the leaflets.

This experiment was repeated in much the same manner on November 24, 1942, and the disease again reproduced. The typical lesions were apparent 3 days after the inoculations were made. When lesions were very numerous, as along the margins or tips of the leaflets, the tissue was killed. The fungus was recovered from the lesions.

In order to ascertain whether or not other species of vetch also were susceptible to this disease, several species were grown in pots in the greenhouse and inoculated when the seedlings were about 6 to 10 inches tall. The following species were included in the experiment: *Vicia sativa* L. (F.C. 29933, F.C. 16462, F.C. 18808, Selection 7, Willamette, and Alba), *V. grandiflora* Scop., *V. atropurpurea* Desf., and *V. villosa* Roth. *V. angustifolia* was included as a known susceptible species. One pot of each lot, containing about 25 seedlings, was atomized with water as a noninoculated control and placed in a large moist chamber. Another pot of each lot was atomized with a suspension of the fungus spores and likewise placed in the moist chamber. The inoculations were made on January 6, 1943, and the pots were held in the moist chamber for 48 hours. The temperature of the chamber ranged from 18° to 20° C. during this period. When removed from the moist chamber none of the noninoculated controls showed any signs of disease, but lesions were evident on many of the inoculated plants. Infection was obtained on all of the lots tested with the exception of *V. villosa* and *V. atropurpurea*. The appearance of the lesions on the different species varied somewhat but were similar in most instances. The symptoms produced on *V. grandiflora* and *V. sativa* (Alba) are illustrated (Fig. 1, B and C). As shown in the figures, there was more tissue killed in *V. grandiflora* leaflets than in those of *V. sativa* (Alba) or of *V. angustifolia*. The lesions continued to enlarge somewhat after the plants were removed from the moist chamber, but seldom reached over 1 mm. in diameter. Considerable defoli-

ation took place in some lots, but, for the most part, as is often the case under natural conditions, the infection was not heavy.

TAXONOMY

The causal fungus is easily isolated and grows well on all media tried. It does not, however, sporulate well on any of them. Usually fruiting is most abundant at the top of long slants.

The mycelium is abundant, white to grayish in mass, varying from colorless to slightly grayish to brownish under the microscope, 4–11 μ in diameter and the branches often are constricted slightly where joining the parent hypha. The conidiophores are simple or branched and commonly elongate forming several fruiting heads. They are the same color as the mycelium, or slightly darker brown, the ends are enlarged, globoid or elongate, each bearing several spores on short sterigmata. Conidia are gray in mass, singly colorless or grayish to brownish like the mycelium, obovate, pyriform, or elliptical in shape and 13.7–25.2 × 8.4–15.8 μ (av. 18.4 × 11.6 μ) in size. Sclerotia were seen in culture only and were white at first, soon turning black, irregular in outline, often rounded when viewed from above but more or less hemispherical, and 1–4 mm. in largest diameter. The perfect stage was not seen.

There seems to be little doubt that this fungus belongs in the *Botrytis cinerea* group.[3]

DISCUSSION

After this work was nearly completed a reference to what is probably the same or a very similar disease was found in a paper by Wilson[4] on the chocolate spot of broad bean. During his investigations he tested the pathogenicity of the chocolate spot fungus on a number of legumes, among them being *Vicia sativa*. This species of vetch became severely infected as shown in his plate 17, figure 4. He does not describe the symptoms, however, hence it can only be assumed that they were identical with those of the same disease on *V. faba* L. If this be the case, presumably the color of the lesions was chocolate instead of the red characteristic of the disease of vetch studied by the writer. Wilson states further that in Great Britain vetch is reputed to suffer from chocolate spot, citing as authority the list of common names of British plant diseases.[5] The similarity between this disease of vetch and that of *V. faba* and the fact that Wilson found that several forms of *Botrytis cinerea* caused chocolate spot of *V. faba,* suggested the desirability of testing the pathogenicity of the vetch form of *B. cinerea* on *V. faba*. This was done by inoculating several plants with spores from a culture of the *Botrytis* from Augusta vetch. Four days after the inoculations were made red lesions, typical of the leaf spot on vetch, appeared on several *V. faba* leaflets from some of which the fungus was recovered. The lesions were slightly darker in color on *V. faba* than on the vetch species. The fact that this form of *B. cinerea* from vetch also attacked *V. faba* is of interest in view of the fact

[3] This conclusion was confirmed by Prof. H. H. Whetzel, Cornell University, Ithaca, N. Y., who kindly examined a culture of the fungus.

[4] Wilson, A. R. The chocolate spot disease of beans (*Vicia faba* L.) caused by *Botrytis cinerea* Pers. Ann. Appl. Biol. 24(2): 258–288. 1937.

[5] British Mycological Society. List of common names of British plant diseases. Trans. Brit. Mycol. Soc. 14: 140–177. 1929. (The disease at that time was attributed to *Bacillus Lathyri* Manns and Taubenh.)

that Wilson found that many forms of *B. cinerea* were capable of causing chocolate spot of *V. faba.*

Having learned that the form of *Botrytis cinerea* from vetch would attack *Vicia faba,* an attempt was made to determine whether the form of *Botrytis* from lupine[6] would also attack vetch. A single inoculation experi- All 4 strains of *V. sativa,* namely Willamette, Selection 7, Alba, and F.C. 18808, became infected. Purple vetch proved to be resistant, just as it was to the form of *Botrytis* from Augusta vetch. In this single test the form of *Botrytis* from lupine did not appear to be quite so pathogenic to vetch as did the form originally from vetch; although this may have no significance, since the environmental conditions and possibly other variable factors undoubtedly were not identical. The fact that all 3 of these hosts, vetch, lupine, and broad bean, are susceptible to these forms of *Botrytis* suggests that these crop plants should not be used in a rotation designed to control the Botrytis disease of any one of them.

Since the original collection of this disease on *Vicia angustifolia* was received from Gainesville, Florida, the same disease has been collected on this host and on *V. sativa* at Quincy, Florida, and also on *V. sativa* at Albany and Experiment, Georgia. Although the leaves were in some cases badly spotted the plants had made a good growth and the damage caused by the disease was slight.

CONTROL

Our knowledge of this disease indicates that for the present at least control measures are not necessary. Rotation should be helpful in preventing the rapid accumulation of inoculum in the field. Inoculation experiments indicate that Smooth and Purple vetches are highly resistant to the disease. *Vicia faba* and *Lupinus angustifolius* L. are susceptible, and, therefore, are not suitable for planting in a rotation designed to control this disease.

SUMMARY

A disease of vetch found in the Southeastern United States caused by a fungus of the *Botrytis cinerea* group is described. Small dark red spots are produced on the leaflets, stems, petioles, and tendrils of several species of the host. *Vicia villosa* and *V. atropurpurea* appeared to be resistant, while all strains of *V. sativa* tested, *V. angustifolia, V. grandiflora,* and *V. faba,* were susceptible. A form of *B. cinerea* from lupine also caused the leaf spot on vetch.

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EXPERIMENT, GEORGIA.

[6] Weimer, J. L. A Botrytis disease of lupines. Phytopath. 33: 319–323. 1943.

ment was conducted, using the same methods as those described previously.

INVASION OF WATER-SOAKED TOBACCO LEAVES BY BACTERIA, SOLUTIONS, AND TOBACCO-MOSAIC VIRUS[1]

STEPHEN DIACHUN, W. D. VALLEAU, AND E. M. JOHNSON

(Accepted for publication August 23, 1943)

Previous reports (1, 3, 5) have shown that water-soaking of tobacco leaves enables leaf-spot bacteria (*Bacterium angulatum* and *Bact. tabacum*) to enter the leaves. Apparently, invasion occurs by way of water channels, presumed to exist between the outer leaf surface and the intercelluar spaces, through stomatal openings. It is not known whether the bacteria enter the leaf by virtue of their own active motility, *i.e.*, by "swimming," or by action of some other force. Knowledge concerning this point is of interest in studies on overwintering of the leaf-spot bacteria in soil (4) and on roots (7). In the method used to determine whether the bacteria are present in soil or on roots, a water suspension of the material to be tested is placed on water-soaked leaves of living plants. If infection occurs, it is concluded that the pathogenic bacteria are present. However, if infection does not occur, it may mean that pathogenic bacteria are not present at all, or that they are present but in an inactive or nonmotile state in which they may not be able to gain entrance. Thus, it is necessary to know whether active motility is essential for leaf invasion. The object of this report is to present indirect evidence indicating that motility is not necessary for bacterial entrance into leaves.

INVASION OF WATER-SOAKED TISSUE BY NONMOTILE BACTERIA

If nonmotile bacteria can invade water-soaked leaves as readily as motile organisms, the implication is that active motility is not necessary, and perhaps that any bacteria may be able to enter leaves without having to swim in. The following test was made to determine whether nonmotile bacteria can invade water-soaked tobacco leaves.

An area of a leaf was water-soaked by forcing a stream of water from a hypodermic syringe against the lower surface. A 24-hour beef-peptone-broth culture of *Staphylococcus aureus* diluted 1–10 with sterile water was poured on the lower surface of the water-soaked area. The area was marked, and, after water-soaking disappeared (30 minutes), was cut out, surface-sterilized 30 seconds in 1/1000 $HgCl_2$, and rinsed in 3 changes of sterile water. A disc was cut out with a sterile cork borer (7 mm. diameter), crushed in 10 cc. of melted potato-dextrose agar, and poured into a Petri plate. Five disc samples were thus tested. Within a few days thousands of colonies of *Staph. aureus* developed on each plate. In the same way, 5 disc samples were tested from a non-water-soaked area of the same leaf

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

inoculated with *Staph. aureus*. Not a single colony of the organism appeared on the plates.

Another area of the same leaf was water-soaked at the same time and inoculated with *Bacterium angulatum*. Sample discs were tested in the same way. Thousands of colonies developed on each plate prepared from tissue water-soaked when inoculated, while no bacteria were recovered from a non-water-soaked inoculated area. This test was repeated 3 times with similar results (Table 1).

The cells of *Staphylococcus aureus* are believed to be nonmotile, while those of *Bacterium angulatum* are motile rods. This test shows that a motile organism has no advantage over a nonmotile organism in entering water-soaked leaf tissue. It does not prove that *Bact. angulatum* enters without swimming, but suggests that it can do so.

TABLE 1.—*Recovery of motile and nonmotile bacteria (Bacterium angulatum and Staphylococcus aureus) from within tobacco leaves inoculated by pouring bacterial suspension on water-soaked tissue*

Organism	Leaf treatment	Number samples tested	Approximate number of colonies on agar from within crushed leaf samples 38.5 mm.² in area		
			Test 1[a]	Test 2	Test 3
Staph. aureus	Water-soaked	5	5,000 to 20,000	1,000 to 3,000	20 to 100
" "	Non-water-soaked	5	0	0	0
B. angulatum	Water-soaked	5	2,000 to 10,000	2,000 to 5,000	150 to 800
" "	Non-water-soaked	5	0	0	0

[a] In Test 1 the 24-hour broth culture used as inoculum was diluted 1 to 10 with water; in Test 2 the dilution was 1 to 100; in Test 3 the dilution was 1 to 1000.

ENTRANCE OF INDIA INK INTO WATER-SOAKED LEAVES

To determine whether nonliving particles can enter water-soaked leaf tissue, India ink was placed on water-soaked areas and adjacent non-water-soaked areas of the same leaf. Invariably the ink entered only the water-soaked areas, producing a blackening of the leaf, visible through both upper and lower surface, that could not be washed off. Entrance apparently was instantaneous, for, even when the ink was washed off immediately after being put on, blackening occurred. Examination of strips of lower epidermis showed accumulation of ink in the stomata. Free-hand sections of leaves showed ink to be confined principally to the surface of spongy cells. The ink in this condition apparently is not toxic to the cells.

ENTRANCE OF SOLUTIONS INTO WATER-SOAKED LEAVES

To determine whether solutions of toxic chemicals can enter water-soaked leaves, the following test was made. The left side of 3 leaves was water-

soaked. A 1–1000 solution of $HgCl_2$ was poured onto the lower surface of both left and right sides, and the leaf was then rinsed with tap water. As the water-soaking disappeared, the tissues collapsed and soon became dry. In spots the entire thickness of the leaf was reduced to a thin parchment-like membrane; other regions of the water-soaked areas collapsed only on the lower surface, which then appeared rough and gray, while the upper surface remained normal. The degree of injury seemed to depend on whether the chemical injured the spongy cells only, or both spongy and palisade cells. This experiment was repeated several times with similar results. Similar injury was produced by $CuSO_4$ (1–100) and Bordeaux mixture (3–3–50) applied to water-soaked tissues. Injury in each case was confined to the water-soaked areas.

ENTRANCE OF TOBACCO MOSAIC VIRUS INTO WATER-SOAKED LEAVES

Caldwell (2) has injected leaves of *Nicotiana glutinosa* with tomato aucuba-mosaic virus without producing infection. Johnson (6) has shown

TABLE 2.—*Recovery of tobacco mosaic virus from within tobacco leaves inoculated by placing infectious leaf extract on water-soaked tissue*

Test	Replication	Number of necrotic spots produced on leaf of NN burley plant		
		Rubbed with surface-sterile tissue from		Water-soaked when in contact with virus, without further treatment
		Leaf water-soaked when in contact with virus	Leaf not water-soaked when in contact with virus	
1	1	51	9	0
	2	8	0	0
2	1	54	0	0
	2	58	0	0
3	1	49	0	0
	2	35	0	0

that water-soaked leaves of an *N. glutinosa* × *N. tabacum* hybrid do not become infected when sprayed with tobacco-mosaic virus. The following tests show that tobacco-mosaic virus placed on the surface of water-soaked leaves enters the leaves but does not cause infection.

An interveinal area of a leaf of a *Nicotiana digluta* × *N. tabacum* hybrid containing the N factor was water-soaked, and immediately leaf extract from a mottled mosaic-diseased burley plant was placed on the lower surface of this area. After the water-soaked condition disappeared (30 minutes), the area was tested to determine whether virus was present inside the leaf. The area was cut out, washed in concentrated trisodium phosphate 60 seconds to destroy virus on the outer surface, rinsed in running tap water, crushed, and rubbed on another leaf of the same plant. Three days later numerous necrotic spots were present on the rubbed area. A non-water-soaked area, on which infectious juice was placed at the same time, was cut out, washed in trisodium phosphate, rinsed, crushed, and rubbed onto

a leaf. It produced no necrotic spots, showing that virus particles were not carried on the leaf surface.

Infectious juice was placed on a similar water-soaked area without further treatment. Infection did not occur. These tests show that tobacco-mosaic virus can enter leaves through stomata of water-soaked areas without producing infection.

Each test was performed in duplicate, and repeated 3 times (Table 2).

CONCLUSION

The tests reported in this paper show that nonmotile bacteria, as well as virus, non-living particles (India ink), and solutions, can enter water-soaked leaves. Pathogenic leaf-spot bacteria need not be motile or free-swimming to invade leaves.

The fact that chemicals can enter water-soaked tissue and cause injury suggests the possibility that naturally induced water-soaking may play a part in the occurrence of spray injury.

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PHYTOPATHOLOGICAL NOTES

Use of Liquid Culture of Fusarium for Field Inoculation of Cotton.—Field testing of cotton breeding material for resistance to *Fusarium vasinfectum* has been handicapped by difficulty in obtaining uniform infection. Attempts to build up natural infestation in field soils by the addition of artificial cultures, on solid media such as oats, corn meal, etc., and infected cotton stalks have been laborious, cumbersome and not entirely successful.

From infection results in greenhouse tests obtained with liquid inoculum with the blackroot-rot organism by Allison and with Fusarium wilt of cotton by Tharp and by Sherbakoff, it seemed likely that liquid inoculum also might be employed successfully in the field. In the method employed by the senior writer in greenhouse tests at Knoxville, the liquid inoculum was introduced easily and effectively through deep holes in the sand, close to the plant roots. The following adaptation of the method was employed in the field at Knoxville in 1942.

The inoculum was prepared as follows: The fungus was cultured on thin layers of agar in standard Petri plates. Several days later small circular discs of about 3 mm. diameter of the growth were cut out and placed on the wet inside surface of a 500-cc. Erlenmeyer flask containing 300 cc. of nutrient solution,[1] spore side to the glass. This was for the purpose of washing out most of the spores into the liquid, and afterwards the discs were washed down into the flasks. For a two-day period the inoculated flasks were thoroughly shaken every 3 or 4 hours during the day time, and were then used for inoculating an unsterilized solution composed of 1 part of table syrup to 100 parts of water at the rate of 1 flask (300 cc.) to 3 gal. of the sweetened water. The bulk culture was prepared in a barrel. During the preparation of the liquid cultures in the flasks and in the barrel a temperature of about 80° F. was maintained. The culture in the barrel was stirred frequently and at the end of 48 hours was ready for use.

The cotton under test was planted in the field June 22, in hills of 5 seeds each, around a wooden peg 1″ × 1″ × 10″, set about 4″ deep in the ground. On July 15, when the plants were about 2 weeks old, the pegs were removed and one-half pint of the inoculum was poured into the hole. The hole was then refilled with soil. The soil is classified as Decatur silt loam, usually considered unfavorable for the development of wilt.

Observations of the amount of wilt were made at frequent intervals. At the end of the growing season the amount of internal discoloration was determined by cutting the stalks. Results of field inoculation of 23 varieties or strains of cotton are given in table 1. Among these varieties wilt infection varied from 0 to more than 50 per cent. The behavior of the check varieties,

[1] Each liter of nutrient solution contained:
10 grams—Sugar
5 grams—Magnesium sulphate
1 gram —Potassium phosphate
1 gram —Ammonium nitrate

TABLE 1.—*Results of field inoculation of cotton varieties with Fusarium vasinfectum, Knoxville, Tennessee, 1942*

Variety and pedigree	Hills	Plants			Plants with wilt symptoms		
		Total	Healthy	Diseased	Dead or defoliated	Internally discolored	Total
	Number	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
Delfos 719 (339)	15	56	40	16	23.2	5.4	28.6
Delfos 719 (992)	15	44	37	7	13.6	2.3	15.9
Acala 911 (330–1–8–2)	15	52	33	19	30.8	5.8	36.6
Acala 911 (330–1–8–4)	15	54	32	22	33.3	7.4	40.7
Trice (2–6)	15	53	37	16	20.7	9.4	30.2
Cook 307 (Resistant check)	12	19	19	0	0.0	0.0	0.0
Acala 911 (330–1–1–8)	15	54	26	28	38.9	13.0	51.9
Coker 33–12 (289–1)	15	48	38	10	20.8	0.0	20.8
Coker 33–12 (289–3)	15	50	24	25	44.0	6.0	50.0
Coker Wilds (176)	15	45	24	19	37.8	4.4	41.2
Delfos 9252 (182)	15	53	34	14	18.9	7.5	25.4
D.P.L. 11A (259)	15	38	28	9	15.8	7.9	22.7
Stoneville 5 (62)	15	57	32	25	38.6	5.3	43.9
Coker 100 (994)	15	45	25	20	31.1	13.3	44.4
Coker Wilt 100 (Resistant ck.)	15	48	43	4	4.2	4.2	8.3
Seabrook #10 (S.I.) (Res. ck.)	14	25	24	0	0.0	0.0	0.0
D.P.L. 11 (51)	15	51	33	18	27.5	7.8	35.3
D.P.L. 4–8 (50)	14	35	33	2	0.0	5.7	5.7
Stoneville (37–13)	15	50	43	6	8.0	4.0	12.0
Trice × Tidewater (578)	14	40	19	21	42.5	10.0	52.5
Stoneville (17–5–8)	15	51	35	15	21.6	7.8	29.4
Stoneville 37 × Stoneville 5	15	50	42	7	6.0	8.0	14.0
Half and Half (Susceptible ck.)	12	24	14	10	25.0	16.7	41.7

Cook 307, Coker Wilt 100, Seabrook #10 (Sea Island), and Half and Half, closely approximates the expected response, based upon previous inoculation trials in the greenhouse and on common field experience.

Although experience with this method has been limited, it does seem to have real promise for field tests. The cost of the culture medium is negligible, and very little time is required in preparation of the inoculum and in making the field inoculations. Results indicate that the method gives a reliable measure of the resistance of the cottons to fusarium wilt.—C. D. SHERBAKOFF, PAUL R. MILLER and D. M. SIMPSON, Tennessee Agricultural Experiment Station, Knoxville, Tenn.

Nicotine Fumigation Injury in Biloxi Soybean.[1]—The leaves of Biloxi soybean plants, which were used in a series of photoperiodic studies over a

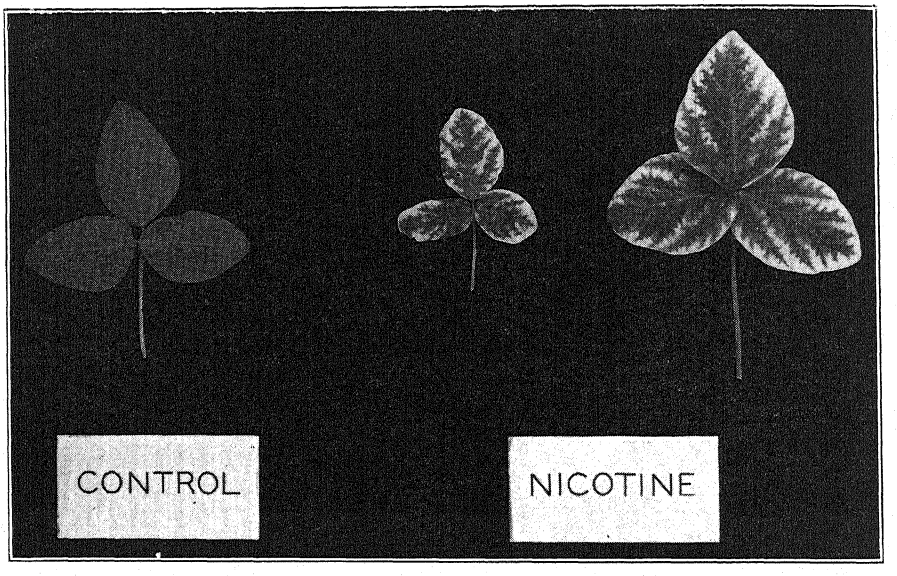


FIG. 1. Nicotine fumigation (right) causes severe and permanent chlorosis in the leaves of Biloxi soybean.

period of several years, frequently exhibited a severe permanent chlorosis, which appeared at the leaf margins and interveinally, occurring at all seasons of the year. Occasionally the injury was so severe that it was necessary to discard the plant material. The plants were grown in a greenhouse in subirrigation nutrient-solution culture, and it was thought at first that a nutritional unbalance or that unfavorable temperature conditions might be responsible for the symptoms. However, variations in nutrition and variations over a wide range of air and root temperatures failed either to produce the symptoms or to cause them to disappear. Finally, it was noticed that the chlorosis invariably followed nicotine fumigation. Therefore, the following investigation was made.

[1] Journal Paper No. 121 of the Purdue University Agricultural Experiment Station.

Test plantings were made in each of two greenhouse units. In one unit, the house was fumigated with a pressure fumigator, using a nicotine fumigant, containing not less than 14 per cent of nicotine, at the rate of one pound per 20,000 cubic feet of air space. Care was taken to make certain that the fumigant was not ejected directly on the plants. The ventilators were opened at the end of 3 hours, but the fumigation was done with outside-air temperature above freezing so that most of the fumigant escaped through the imperfectly sealed glass before the end of the fumigation period. No fumigation was done in the second greenhouse unit.

Within 24 hours after the treatment, the fumigated plants began to show marginal chlorosis on the young unfolding leaves and in about 4 days the typical marginal and interveinal chlorosis was manifest (Fig. 1). The photograph was taken 30 days after fumigation. The rapidity of development of the symptoms depended on the rate of growth. Where rapid growth occurred, the symptoms appeared more quickly. The injury was confined largely to the young, developing leaves. Those within the bud showed little injury. If the older leaves were damaged, the chlorosis usually was not so severe in them and the injured leaf tissue became brown and necrotic, usually in 10 days to 2 weeks. Subsequent fumigations made without the use of the pressure fumigator have resulted in injury similar to that described above.

While the writers have assumed that the injury is due to nicotine, it is entirely possible that it was caused by products derived from combustion accelerators or to other products of the fumigant.—ALICE P. WITHROW and J. P. BIEBEL, Department of Horticulture, Purdue University, West Lafayette, Indiana.

Cercospora Fruit and Leaf Spot of Olive.—In May, 1941, a grower in Solano County, California, called our attention to olive fruits that had remained on the trees during the winter without normal blackening over the entire surface. The stem half of the fruits had remained green, except for scattered purple spots. Stained radial sections through these spots revealed that the tissues beneath were heavily infected by very small hyphae that grew between the host cells, but did not enter the cells and did not kill them.

In November, 1941, W. T. Horne and I. J. Condit sent us olive fruits from Southern California that showed similar purple spots on green fruits (Fig. 1, A). Sections and cultures from these fruits revealed the same fungus observed in the olives from Solano County.

The fungus is very slow-growing in culture (Fig. 1, B) and may, therefore, not be detected by the usual isolation technique. After the fungus has been in culture for some time it produces small pycnidiumlike structures (Fig. 1, C, a) containing minute unicellular spores, which we have not been able to germinate. It appears probable that these spores may be spermatia (Fig. 1, C, b). Multiseptate conidia are later produced in culture.

Infections on leaves may not be detected unless a careful search is made. They result in the formation of indistinct darkened areas on the lower sur-

face of the leaves and may cause early drop of infected leaves. The imperfect fructifications of this fungus have been observed on the discolored spots on overripe fruits (Fig. 1, D) and growing from stomata on the lower surface of leaves. The spores are similar to the multiseptate conidia produced in agar cultures, but are slightly shorter when growing on the host.

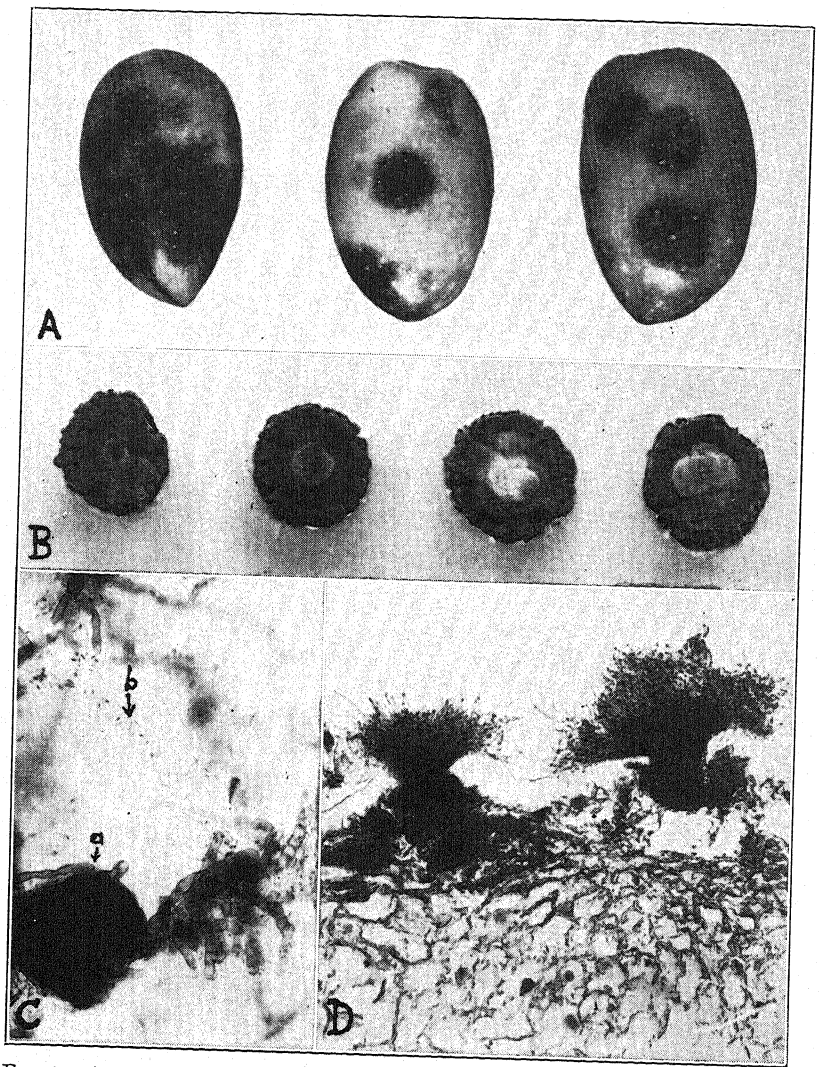


FIG. 1. A. Symptoms of Cercospora infections on olive fruits. B. Fourteen-day-old colonies of fungus on potato-dextrose agar. C. Spermogonium (a) and spermatia (b). D. Section through overripe olive fruit showing conidial tufts breaking through the cuticle.

The size and septation of the spores are similar to those of *Cercospora cladosporioides* Sacc. reported by Saccardo on olive leaves in Italy and Algeria. It, therefore, appears probable that we are dealing with the same species described by Saccardo.

In California this fungus has caused significant damage to fruits that are to be pickled as green olives. R. H. Vaughn of the Fruit Products Division, University of California, has found that infected fruits show the discolored spots after pickling and must be discarded. However, infected fruits are satisfactory for pickling as ripe olives. After the ripe-olive pickling process the infected, as well as the uninfected, portions of the fruits assume the same desired dark color. The flavor and texture of the fruits are apparently not impaired by the fungus, and no evidence of toxicity has been noted by individuals who have eaten large quantities of the ripe pickled fruit.—H. N. HANSEN and T. E. RAWLINS, Division of Plant Pathology, University of California, Berkeley, California.

New Strain of Agrobacterium rubi from Boysenberry.—The first serious outbreak of cane gall on boysenberry, *Rubus ursinus* var. *loganobaccus*,[1] thus far noted by the writer occurred in a planting near Auburn, New York, in the spring of 1942. The cultures obtained from these galls (Fig. 1, A) were studied bacteriologically and appeared identical in growth character, morphology, and physiology to those previously reported by the writer[2] and to those isolated about the same time as the above from black raspberry, *R. occidentalis* L., collected in a local garden.

Pathogenicity tests with cultures from both sources made into new black raspberry canes in midsummer all behaved similarly in inducing galls at the points of inoculation. When the galls thus induced on the new canes were removed by pruning the canes at variable distances below the position of the galls in late October, new galls appeared on the majority of canes the following season. These galls occurred in cases where the pruning cuts had been made as much as 8 to 12 inches below the gall. This is positive evidence that the cane-gall organism was able to migrate through first-year canes late in the season as they approach dormancy.

In making a comparative study with two other cell-stimulating organisms, *Agrobacterium tumefaciens*[3] and *A. rhizogenes*, on Bonny Best tomato, Cumberland black raspberry, and *Kalanchoe daigremontiana*, it was discovered that on wound inoculation the boysenberry strains, but not those from black raspberry, stimulated tiny galls (2 mm.) on *Kalanchoe* (Fig. 1, B) within 1 month. Reisolations demonstrated the presence of a pathogen identical to the original in the tiny galls. Excision of the tiny galls resulted in the reappearance of galls at the original sites of inoculation

[1] BAILEY, L. H. Species Batorum. The genus Rubus in North America. Gentes Herbarum. 5(1): 1–64. 1941.

[2] HILDEBRAND, E. M. Cane gall of brambles caused by *Phytomonas rubi* n. sp. Jour. Agr. Res. [U.S.] 61: 685–696. 1940.

[3] The taxonomy of the cell-stimulating pathogens, of which *Phytomonas tumefaciens* (Sm. and Town.) Bergey *et al.* is the type species, has been recently changed by Conn (Conn, H. J. Validity of the genus Alcaligenes. Jour. Bact. 44: 353–360. 1942) to the new genus *Agrobacterium*. This designation for the cane gall organism, *Agrobacterium rubi*, was first used by Starr and Weiss (Starr, M., and J. E. Weiss. Growth of phytopathogenic bacteria in a synthetic asparagin medium. Phytopath. 33: 314–318. 1943.

(Fig. 1, B). Reinoculations into *Kalanchoe* again resulted in tiny galls. Because only the boysenberry strains of the cane gall organism were capable

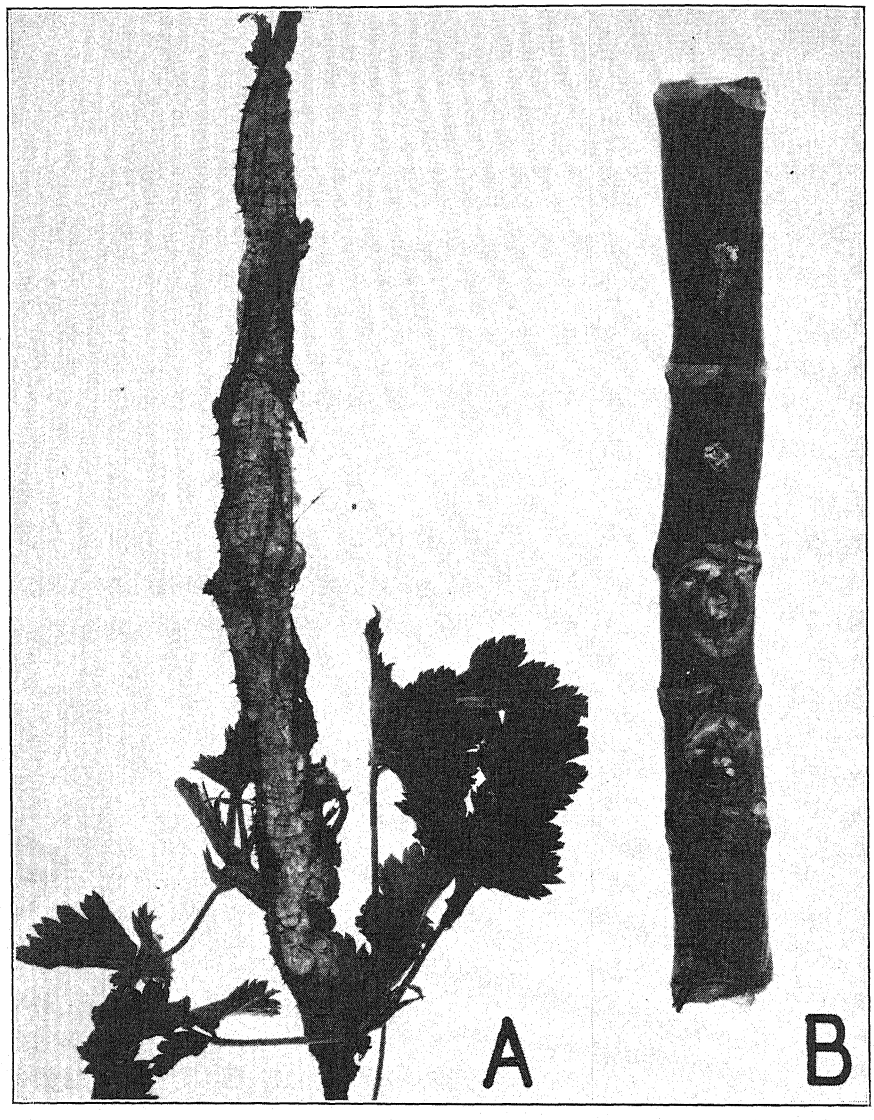


FIG. 1. A. Symptoms of cane gall, occurring naturally on boysenberry and completely involving a section of stem about 1 foot long. $\times \frac{1}{2}$. B. Tiny galls induced on Kalanchoe by a boysenberry strain of cane gall organism 5 months after inoculation. The two lower galls were excised a little over a month before the photograph was made and were replaced by the galls shown. $\times \frac{3}{4}$.

of inducing tiny galls on *Kalanchoe*, they apparently represent a new pathogenetic strain, which extends the host range of this organism beyond the genus *Rubus*.—E. N. HILDEBRAND, Cornell University, Ithaca, N. Y.

BOOK REVIEW

DODGE, BERNARD O., and HAROLD W. RICKETT. *Diseases and Pests of Ornamental Plants.* xi + 638 p. illus. (figures not serially numbered). The Jaques Cattell Press, Lancaster, Pa. 1943. $6.50.

Although several experiment station bulletins dealing in general with the diseases and pests of ornamental plants have been issued in the United States in recent years, and although limited parts of this field, especially that of ornamental trees and shrubs, have been covered in at least two commendable manuals, the book under review is the first to attempt the whole range of ornamentals and to cover all manner of ailments and pests. Since the subject matter of the ornamental plant field is so heterogeneous—some one somewhere probably has regarded almost every kind of plant that grows as having decorative value—the task of compiling the relevant disease and pest information is truly formidable. When to this difficulty is added that of an extreme disparity in the value attaching to the currency that circulates in this realm—some of it sound and much of it otherwise, because floriculturally-minded persons, although voluminous writers, have not been until recently very scientific—it follows that standards of thoroughness and accuracy to be expected in books dealing with the pathology of economic crops cannot yet be demanded here.

This book may be appraised for (1) its value as a handbook for gardeners whether amateur or commercial, and (2) as a reference book for professional plant pathologists (including entomologists!). For amateur gardeners it brings together in convenient form a large amount of information that is otherwise inaccessible or is widely scattered in bulletins, periodicals, and other books. The subject of disease and pest control is presented with thoroughness and on the whole with up-to-date information, reflecting the senior author's experience with a wide variety of plants under garden and greenhouse conditions. Somewhat less familiarity is indicated with the pathological problems of commercial culture of certain ornamentals, flowering bulbs for example, in the statements, p. 424 *et seq.* (1) that basal rot is not serious on the trumpet type of narcissus, whereas it is by far the most important disease of commercial narcissus culture, consisting largely of trumpet types, in this country; (2) in the recommendations of long soaking treatments for the control of basal rot, whereas commercial growers have found only short dip treatments (which are not mentioned at all) to be practicable; (3) the vector of narcissus mosaic is not known, whereas a number of aphid species have been shown to transmit this virus. Other cases in point are omission of the highly injurious pests of *Gerbera,* the cyclamen and bulb mites under greenhouse culture, and root knot in both greenhouse and nursery plantings; in omission of all reference to the Heteroceras spot of cultivated *Nymphaea;* the statement that *Sclerotium rolfsii* is not reported on tulips in America, whereas this fungus, or its form *S. delphinii,* causes an important field disease of commercial tulip culture in both Long Island and North Carolina.

Professional workers in this field will find the assemblage of pathological and entomological information very useful, although there are some important omissions, and other items are included apparently on the sole basis of their mention in European literature without reference to their occurrence or importance here.—FREEMAN WEISS, U. S. Bureau of Plant Industry Station, Beltsville, Maryland.

FUNGI FOR PENICILLIN PRODUCTION

A project is being organized at the University of Minnesota Agricultural Experiment Station, Division of Plant Pathology on Botany, to survey Penicillia belonging to the *Penicillium notatum* group and also species of *Aspergillus* for the production of penicillin. The project is under the supervision of Dr. E. C. Stakeman. Cultures of organisms are desired, and individuals are requested to forward isolations of the groups of fungi mentioned to the laboratory indicated. Isolations known to produce penicillin are especially desired.

Individuals who wish to survey other fungi for penicillin activity can obtain directions for a standard technique from the United States Department Agriculture, Regional Laboratory at Peoria, Illinois.

ALBERT L. ELDER
War Production Board
Coordinator of Penicillin Program

A VASCULAR DISEASE OF GLADIOLUS CAUSED BY FUSARIUM

LUCIA MCCULLOCH[1]

(Accepted for publication July 17, 1943)

INTRODUCTION

A Fusarium disease of gladiolus commonly known as yellows, wilt, or core rot, scarcely known and certainly not serious in 1920, has now spread to most if not all producing regions in the United States and is generally considered the most serious disease of gladiolus. The Fusarium wilt disease, deep seated in the interior of the corms, easily escapes notice. Since symptoms become visible at the surface of the corm only in advanced stages of the disease, infected corms are often shipped and planted without any suspicion of their condition.

The present publication is based upon 18 years of investigation of various phases of the disease.

HISTORY OF THE DISEASE

In 1923 a large number of gladiolus corms of the Nanus type (Bride, Blushing Bride, Peach Blossom, Ruby, Rosy Gem, San Mateo, and others) were received from two localities in California. Although the corms were clean, bright, and normal in exterior appearance, they showed on cutting 90 per cent interior rot (Fig. 1, *A*, *B*). The rot varied from a slight discoloration in the basal scar to browning of the entire core and radiating fibro-vascular strands. From these corms a *Fusarium* was isolated. From later shipments from the same localities the same *Fusarium* was consistently isolated, and inoculation experiments proved that this organism was capable of producing an internal rot of Nanus varieties and similar symptoms in the Primulinus and Grandiflorus types.

In the following year, Nanus, Primulinus, and Grandiflorus types of gladiolus affected with the same vascular necrosis were received from several western areas and in 1925 and 1926 similar specimens came from States as widely separated as North Dakota, Mississippi, and New Jersey. The progress of the disease seemed definitely from the West with prevalence increasing each season. Two of the California growers stated in 1923 that for several years they had imported stock from Holland. In 1926 the writer found the disease in the variety Odin in a shipment from Holland. Again in 1927 in another shipment from Holland the variety Odin and a Nanus type, Bride, had typical symptoms of the disease. From all of these specimens the usual *Fusarium* was isolated.

In the literature there are numerous references to Fusarium diseases of gladiolus but with descriptions not always sufficient for identification. The earliest reference found by the writer that seems to describe the disease

[1] Formerly associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

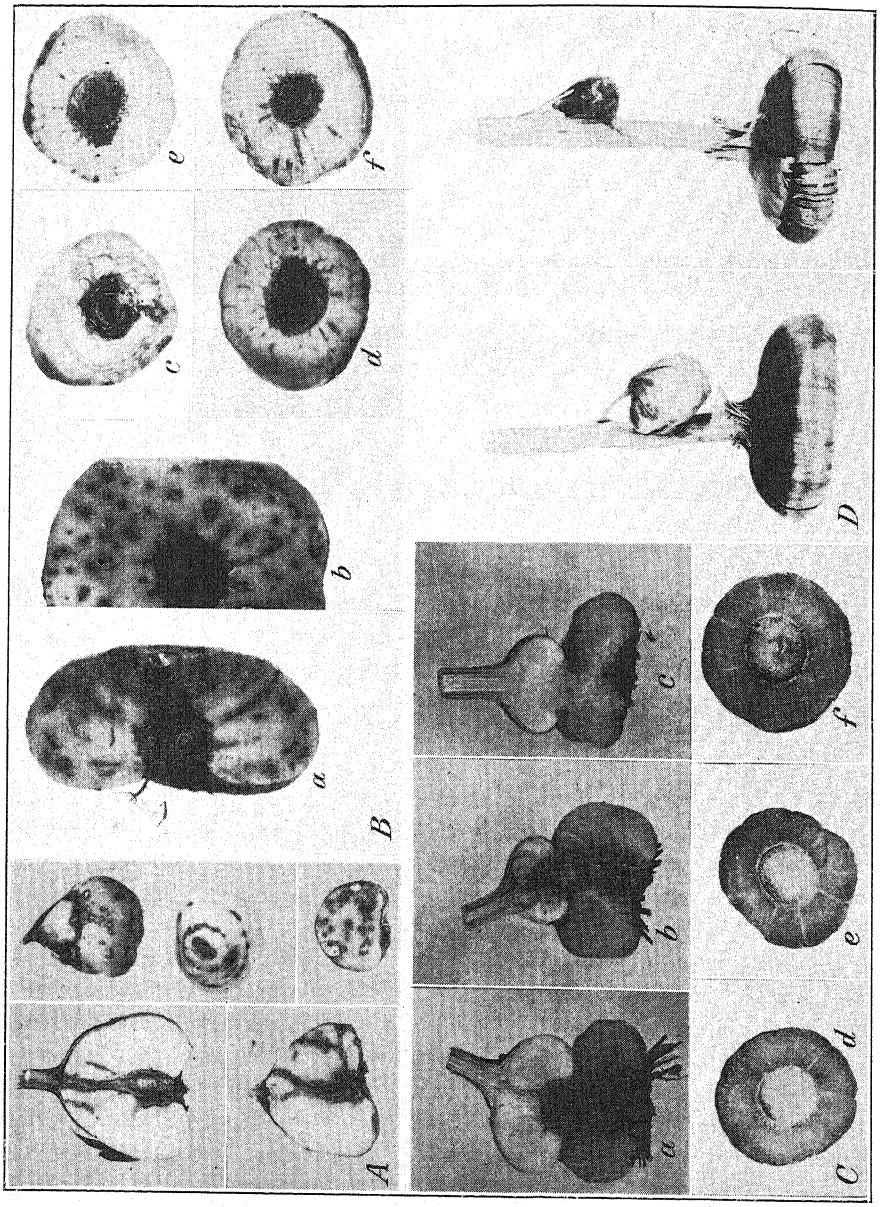


FIG. 1. Vascular infection of gladiolus corms. *A*. Nanus varieties: longitudinal and transverse sections and exterior of *Fusarium*-infected corms from California in 1923. *B*. *a*, Longitudinal; *b*, transverse sections of a corm from storage in midwinter; *c*, base of an infected corm; *d–f*, transverse sections of *c* at different levels. *C*. *a* and *b*, Extension of infection from the parent corm into the new corm; *c*, healthy corm; *d*, slightly infected cylinder; *e*, nearly half of vascular cylinder infected; *f*, entire cylinder infected. *D*. Aerial corms collected in October on current growth. All × ½ natural size.

clearly is a note by a California grower, W. A. Pryal (18), who described an interior corm rot and leaf yellowing of gladiolus in 1909. Massey, in 1922 (7), published a brief note, and, in 1926 (8), a full description of a Fusarium decay of gladiolus corms. Van Poeteren (25) reported the vascular disease as present in Holland as early as 1925. A dry rot of gladiolus caused by *Fusarium* was reported from England in 1927 (2), and a vascular Fusarium disease in 1939 (13). The vascular type was reported from California in 1927 (20). Later accounts of the vascular disease are found in reports by Bellard (3), Dimock (4), Nelson (14, 15, 16, 17), the writer (9, 11), and others (1).

SYMPTOMS

Leaf Symptoms

In infected Nanus gladiolus, apparently normal plants suddenly wilt or turn yellow and die prematurely. In the larger types of gladiolus the stout leaves do not wilt easily and wilting is less noticeable, often absent. The falling over of the whole plant is due more to destruction of tissues at or near the ground level than to wilting from lack of water.

When infected stock is planted, the more seriously diseased corms rot, without sprouting, or produce feeble shoots that soon die. Slightly infected corms often grow fairly well, some even producing a new corm. When healthy stock is planted in infested soil, all or most of the corms germinate and the plants for a time appear normal but soon show stunting and loss of color. With either infected stock or infested soil the end results of stunting, yellowing, and drying of the foliage are similar. The roots of such plants show various degrees of discoloration and rot. In advanced stages of disease the roots are absent or reduced to the central vascular strand. In the corms the infection may be slight and only in the basal scar or the entire core and vascular strands (Fig. 1, *B, C*) may be decayed.

If the plants are fairly well grown before the infection becomes serious, the leaf yellowing is correspondingly delayed or even absent. In young, tender leaves, wilt may occur before the leaves yellow. With a uniform infection of the whole vascular system, all of the leaves become yellow and dry, the change usually beginning at the tip and proceeding regularly downward. Sometimes leaf yellowing shows first in the lower leaves, and in some partially resistant varieties a slight wilting and yellowing of the first leaf is the only indication of infection. Corms infected in only a section of the root plate show correspondingly less yellow in the leaves. Probably the yellowed leaves are in vascular connection with infected roots, but it is difficult to establish this.

In very susceptible varieties the foliage symptoms appear very early. In heavily infested soil and with adverse cultural conditions, even the resistant varieties sometimes become slightly infected, but such infection produces very slight evidence of disease in the corms and usually no noticeable foliage symptom. Intermediate between the very susceptible and the resistant

varieties are those in which more or less infection occurs without disease symptoms in the leaves.

The leaves of some varieties (Hopi, Los Angeles, Maid of Orleans, Alice Tiplady, and others) turn yellow naturally with maturity, with appearance very similar to yellows, but examination of the roots and corms shows no trace of disease. Furthermore, diseases other than Fusarium yellows may cause yellowing, so that this is not an entirely dependable symptom.

Corm symptoms

In corms the disease symptom varies from a slight discoloration at the base to complete rot. Extensive interior rot may exist without any outward sign of disease. When infected plants are harvested, the new corms may appear entirely normal, but, when cut, usually show a surprising amount of brown rot in the interior (Fig. 2, *A*). In typical average stages of disease both the parent and the new corms have discolored cores and radiating vascular strands. In a more advanced stage the infected strands reach the surface of the corm at the nodes where brown lesions develop (Fig. 2, *E*, *a–b*). If this stage of infection develops before the corm is dug and the husks are not too dry, the infection spreads into the vessels of the husk (Fig. 2, *E*, *c*). The node lesions are more often found in corms in storage. These surface lesions are bright dark-brown, with margins of light reddish-brown. However, there is more or less variation in the different varieties of gladiolus.

Infection may exist in a slightly broken basal scar, perhaps with no discoloration. Sometimes the parent corm shows less disease than the new corm. In severe infections, especially if the soil is moist, the parent corm is completely rotted by harvest time instead of being normally dry and shriveled. In such cases the new corm is usually in an advanced stage of rot (Fig. 2, *A*). Sometimes new corms are infected directly from contractile roots, while the parent corm remains disease-free.

The diseased vascular tissue varies in color from light to medium brown (Cinnamon buff, and Saccardo's umber in Ridgway (19)). The fungus apparently develops in advance of the discoloration, as it can be isolated from vessels that are still normal in color. The texture of the infected vascular tissue is rather firm, woody, or tough. Under moist conditions the fungus passes beyond the vascular tissues and causes a general, often soft rot of the fleshy part of the corm with various secondary organisms usually present. In plants that have continued growth to or nearly to maturity, the course of the disease is easily traced from the roots through the parent corm into the new corm (Fig. 1, *C*). The exterior of such corms is usually normal. As the tissues dry, small cavities form inside the diseased corms and in these the delicate white hyphae and occasionally the microconidia of the fungus are found.

Microscopic examination of fresh or fixed sections shows fungus hyphae inside the vessels (Fig. 3), abundant in some, rather scanty in others. In the margins of some infected areas the mycelium eluded microscopic obser-

vation, but from similar tissue the fungus could be demonstrated by culturing.

In addition to the typical vascular disease, there is the less typical corm condition variously designated as doughnut, high crown, or hollow core. Beginning at the base there is a progressive browning, drying, and shrinkage

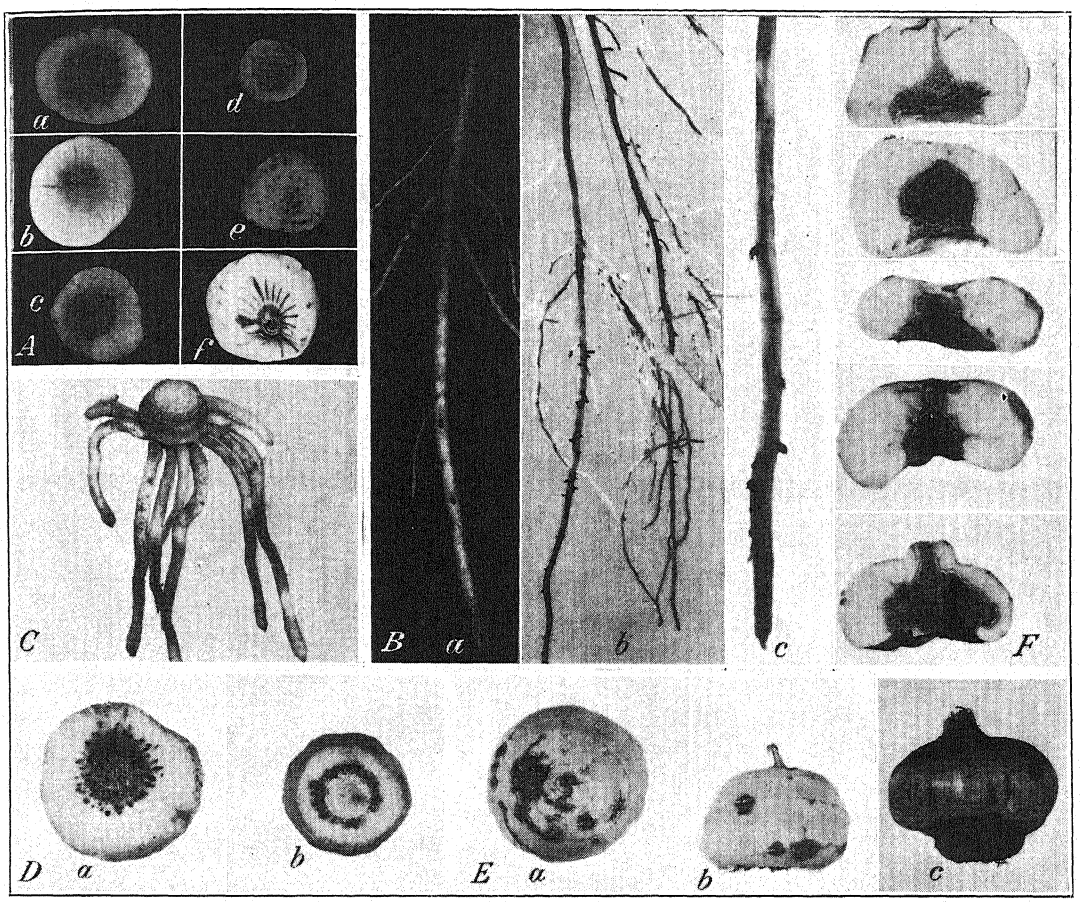


FIG. 2. Vascular infection of gladiolus. *A*. Immature corms: *a–d*, core decay in cross sections; *e*, small lesions along the nodes; *f*, black streaks in husks due to infection. ×½. *B*. Infected fibrous roots. *a* and *b*, grown in sand infested with *Fusarium;* respectively, ×1½ and ×1; *c*, grown in water with *Fusarium* added; the cortex is translucent, showing the dark, infected central vascular strand; ×1½. Note that infected areas are dark. *C*. Infected contractile roots, grown in soil infested with *Fusarium*. ×½. *D*. Base of fibrous roots, blackened by infection: *a*, exterior; *b*, cut across base of corm. *E*. Interior vascular infection breaking through to the surface along the nodes (*a–b*), and extending into the husks (*c*). ×½. *F*. Various stages in the "doughnut" type of rot. ×½.

of the core tissues. Extreme examples have large holes through the center, while the early stages have merely a slight discoloration in the basal scar. Various intermediate stages are found (Fig. 2, *F*). The hole or cavity is usually widest at the base, and in its enlargement may destroy part or all of the root plate. At the top it is ¼ to ½ inch in diameter, sometimes larger,

often including and destroying the terminal buds. The extent of the cavity is limited to the core tissues with occasional slight extensions into the radiating vascular strands. The brown wall of the cavity is thin ($\frac{1}{2}$ to 2 mm. thick), hard, and woody. The outer surface of this wall is smooth or occasionally with thorn-like projections into the sound, fleshy part of the corm. The whole of this dry, hard core can be separated easily and cleanly from the rest of the corm.

The doughnut, as well as the vascular, type of infection begins in the field, and the extent of the rot depends on the length of time between infection and harvesting. In very moist soil the corms may rot entirely. After

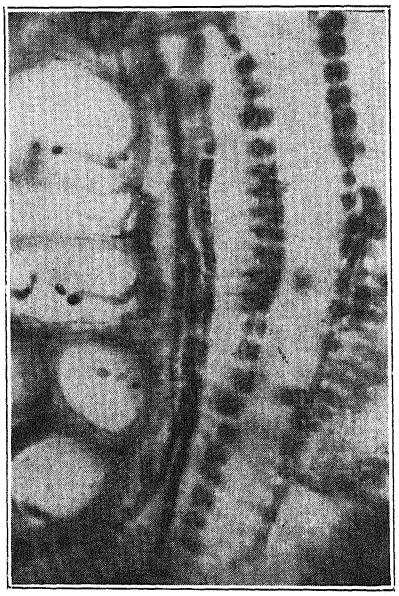


FIG. 3. Stained section from an infected gladiolus corm. Note three hyphae of the *Fusarium* in longitudinal view and a few in cross section. ×1,370.

digging and curing the central part becomes hard and dry and tends to disintegrate and to fall out, leaving a clean, walled cavity or hole from the bottom to the top of the corm. Corms measured when placed in storage and again in the spring showed little or no enlargement of the cavity during storage at 5° to 10° C.

All of the few plants found with small aerial corms (Fig. 1, *D*) had the doughnut condition in the parent corms below ground. These aerial corms were sound and clean in appearance, but no isolation of the fungus was attempted.

The distribution and occurrence of the doughnut type of disease coincides with that of the vascular type. Whether infection results in the vascular or the doughnut condition seems to depend chiefly on the variety, but possibly, also on the age of the corm, the soil, and the virulence of the parasite. The variety Dr. F. E. Bennett is very subject to the doughnut disease. Less

frequently it occurs in Commander Koehl, Mrs. Francis King, Mrs. Frank Pendleton, Halley, Schwaben, E. J. Shaylor, Virginia, and others that are also susceptible to the vascular infection. High crown to doughnut symptoms have been found in a few corms of Picardy, Giant Nymph, and Golden Dream.

Root Symptoms

In fibrous[2] roots the first indication of disease is a rusty color, becoming darker, even black. The infection may occur at any point along the root, but most frequently at the tip. Lateral roots die back, leaving a dark spot on the main root (Fig. 2, *B, a*). Main roots are invaded at their tips, at the bases of laterals, or at any other point. Unless checked, the fungus continues its growth and enters the root plate.

As new roots continue to push out from the root plate, some may escape infection. When, because of disease or injury, the root plate is destroyed, roots often develop from parts above it, even halfway up the side of the corm.

The infected roots soon die, disappear, or are reduced to thin, wiry strands. The dark vascular bundle of the infected root can be seen through the translucent cortex in roots grown in water or wet sand (Fig. 2, *B, b–c*). The proximal ends of the fibrous roots are embedded in the base of the corm to a depth of 3 to 5 mm. or more. These root bases, normally clean and white, are blackened by the Fusarium infection (Fig. 2, *D*).

When all of the root bases are invaded and darkened the parent corm shows a complete dark ring around the core (Fig. 1, *C, f*). If only a few roots in one part of the root plate are diseased, only a part of the vascular ring of the corm is discolored (Fig. 1, *C, d–e*).

Contractile Roots

Contractile roots[3] (description of fibrous roots applies in general to contractile roots), developing comparatively late in the season, seem less subject to infection than the fibrous roots. These roots become infected at any point along their length but most frequently in the thick upper part and particularly on the under side. Lesions of considerable size often escape observa-

[2] Fibrous roots are the long, slender, freely-branching roots that develop at the base of the parent corm shortly after planting. In damp storage these roots often begin growth before planting. In the soil they persist and function as feeding roots until the new corm is matured.

After the foliage leaves are well grown and the new corm has begun to enlarge at the base of the new shoot, contractile roots develop from the nodes at the extreme base of the new corm. These roots are thick, fleshy, white, and more or less transversely wrinkled. They extend deeply into the soil and have few or no branches. Their function seems to be chiefly that of support or anchorage for the plant. Deep-planted corms are less likely to develop contractile roots than corms nearer the soil surface. Stolons, originating from the same region as the contractile roots, develop somewhat later and produce cormels. The exact point of attachment of contractile roots and stolons is not easily determined because of the extremely short neck or stem between the old and the new corms. However, when the two corms are separated in the cleaning process, the dry, shriveled contractile roots and the stalked cormels are most likely to remain attached to the new corm, at least temporarily, and thus indicate their placement. Further details of gladiolus corm morphology are supplied by Geiger (6).

[3] See footnote 2.

tion until the under side of the root is examined. Infection is first visible as a small spot or red or red-brown streak. From this early stage pure cultures of *Fusarium* can be isolated. The lesions (Fig. 2, *C*) become more or less elongated, $\frac{1}{2}$ to $2\frac{1}{2}$ inches long, often confined to one side of the root, or the root may be girdled. In advanced stages the whole root is brown and shriveled. Dissection and stained sections show that the infection starts in the cortex and penetrates into the vascular bundle, which becomes dark, reddish-brown. Infection spreads in both directions and may extend into the vascular tissue of the new corm.

THE COURSE OF NATURAL INFECTION

New corms can be infected directly from infected parent corms or from the soil. From the soil the *Fusarium* may gain entrance through fibrous roots, through contractile roots, or through the leaf bases near or below the soil line.

When fibrous roots are attacked, the hyphae grow upward in the large straight vessels of the root until they reach and enter the root plate and the vascular tissues of the corm. The vessels of the corm are much convoluted but their general direction is upward. Growing in these large vessels, the fungus eventually penetrates into the new corm, where it often causes more decay than in the parent corm.

From infected contractile roots the fungus grows through the vessels to and into the vascular tissue of the new corm, where infection is indicated by a discolored area at the base of the root. Sections show that the contractile roots, like the fibrous roots, extend a few millimeters into the corm, and that the vascular bundle of the root passes into the corm as a compact cylinder, then spreads out fanlike and merges with the vessels of the new corm. It may happen that only one of two or more daughter corms becomes infected from contractile roots.

If infection has originated from both fibrous and contractile roots, the point of origin of infection is difficult or impossible to determine. Infection of either daughter or parent corms can take place from either fibrous or contractile roots, but perhaps only in severe infection of long duration would the parent corm become infected from a contractile root. Infection continues in the cortex of the contractile root and may possibly reach the new corm by this path, as well as through the vascular tissues, but microscopic examination has failed to prove infection from the cortex. Infection may or may not reach the corm before digging time. It is likely that the infection may continue to spread during curing from the drying root into the more succulent tissues of the new corm.

Another point of infection, particularly in moist weather, seems to be along the stem or compacted leaf bases near or below ground level. Infection in these parts progresses downward through the leaf bases to the nodes of the new corm, where small brown lesions develop. These lesions are like those produced by the extension of infection from the interior of the corm,

except that they lack any connection with interior rot. The node lesions originating from stem infection may be shallow or of considerable depth. Such infections have been observed frequently, but not experimentally produced by the writer. Surface lesions caused by this *Fusarium* develop only from infected vascular tissue of corms or leaves.

The progress of the disease and the appearance of leaf symptoms are determined by the extent of the infection in the planting stock or the degree of infestation in the soil, or both, also on the variety of gladiolus, and the temperature and moisture conditions. Weather conditions have considerable effect on the progress of the disease. Low air and soil temperatures retard the development of the *Fusarium,* while very moist soil and high temperatures accelerate its growth and also favor secondary organisms that hasten the destruction of the corm.

When diseased corms are planted in clean soil they regularly yield a diseased crop or none, and furthermore, contaminate the soil. Corms with very slight infection occasionally outgrow it and produce good plants and clean new corms.

In undisturbed soil the fungus seems not to spread rapidly or far in one season. Susceptible varieties of gladiolus have reached maturity in perfect health when grown 6 inches from others so seriously infected that they died before blooming.

The presence of the fungus can be demonstrated by isolation from any point along the diseased root, at its base in the corm, or in the infected area in the corm. The course of infection is easily followed because of the discoloration caused by the fungus. In both free-hand and stained sections of recently infected roots and corms the hyphae of the *Fusarium* are found only in the tracheae. Some tracheae are full of the strands of hyphae, others show few or none (Fig. 3).

ISOLATION AND PATHOGENICITY OF THE FUSARIUM

Isolation of the Pathogen

Hundreds of isolations during the past 18 years have demonstrated the constant association of a certain type of *Fusarium* with the typical vascular disease of gladiolus. The greater number of these isolations were made from plants grown and experimentally infected in the course of a study of the disease. In addition, isolations were made from infected corms of many varieties that were received from practically all of the gladiolus-growing regions of the United States and a few from Holland.

From recently attacked corms and especially from the developing new corm infected directly from the parent corm, the *Fusarium* is easily isolated in pure culture. In many cases the *Fusarium* was isolated from several parts of the same corm, the core, the radiating vascular bundles, the husks, and from node lesions when any were present.

The pathogen has been isolated from both fibrous and contractile roots and from the base of these roots. It has been isolated from the stem or com-

pacted bases of leaves and flower stalk as much as 6 inches above the ground level. While there are certain very typical symptoms in most cases of this vascular disease of gladiolus, there are also some differences due to the variety, to the stage of infection, and to the conditions of growth or storage. Isolations were made from all such unusual or doubtful specimens.

Under the usual growing or storage conditions the *Fusarium*-infected corms are soon invaded by one or more secondary organisms. Because of the presence of these secondary organisms, the isolation of the causal *Fusarium* from advanced stages of the doughnut type, especially from the cured, stored corms, is difficult.

Every effort was made in isolations and transfers to secure and maintain only pure cultures and nothing has developed to cast any doubt on the purity of the majority of the isolates. However, to satisfy the demand for data obtained from single spore cultures, many such isolations have been made. Spores from a culture were dispersed in water and diluted until there were only 10 or 12 spores in a large drop. Such a dilution was spread over the surface of a hard-agar plate. With only 5 cc. of clear agar in a plate, the spores are easily seen in microscopic examination through the bottom of the plate. When the spores germinate they are cut out, placed on a slide, and again examined before transfer to a tube as a single spore. For most isolates, with few or no macroconidia, microconidia were used for the single-spore isolations. Several single-spore isolates from each of 22 isolates were checked for pathogenicity and cultural and morphological characters.

Methods of Inoculation

The most reliable and natural method of inoculation is to plant corms in sterilized soil or sand to which the pathogen has been added at, or shortly before, planting time.

The *Fusarium* for inoculation purposes was usually grown on steamed oats, sometimes on rice, potato, or other media. When well developed the growth was finely divided and measured amounts thoroughly mixed with the soil in the lower part of the pots or bench. The corms were placed on this inoculated soil and covered with several inches of clean soil.

In none of the inoculation experiments did the *Fusarium* affect the intact epidermis of corms, even when the inoculum was placed directly on the corm. Some wounded areas developed decay varying from small, shallow brown spots to rather extensive rot, sometimes reaching and entering the vascular tissues.

When Fusarium cultures, crushed and diluted in water, were poured over plants nearing maturity, the fibrous roots and the bases of the parent corms became infected, but the disease spread into very few of the new corms. Possibly older plants are somewhat resistant, or the fungus did not have time to invade all parts of the plants. In comparable trials on the same varieties of gladiolus, inoculum added to soil at planting time resulted in 85 to 100 per cent infection, while that added to soil 8 weeks after planting produced only 40 to 70 per cent infection.

To observe the effect of inoculation on roots, corms were grown over sterile tap water. In sterile water the roots remained clean and free from disease but those in water containing *Fusarium* soon discolored and rotted (Fig. 2, *B, c*). On isolation from such roots, *Fusarium* and often also bacteria and *Penicillium* were recovered. Clean, sterile, wet sand proved a good medium for root inoculation experiments, as frequent examinations could be made without injury to the roots.

Mature leaves of gladiolus were inoculated in several tests. When the inoculated area was kept moist, a general rot with only slight indication of vascular infection developed in which *Fusarium, Penicillium,* and bacteria were found. If the inoculated area was exposed to the usual atmospheric conditions, no infection resulted.

While a large number of varieties have been used in inoculation tests, those most frequently used were Mrs. Francis King, Mrs. Frank Pendleton, Dr. F. E. Bennett, Mrs. Dr. Norton, Tyco Zang, E. J. Shaylor, Crimson Glow, Schwaben, Alice Tiplady, Giant Nymph, and Tyrian Beauty. It was only by chance that most of the early tests were made with susceptible varieties.

Pathogenicity

In the beginning of this study an effort was made to test the pathogenicity of each organism isolated from corms received for examination and diagnosis. When, after numerous tests, a certain type of *Fusarium* was recognized as the responsible pathogen, the tests were restricted to isolates from different varieties of gladiolus, from different localities, or from specimens showing atypical symptoms.

For the pathogenicity tests, selected corms were carefully examined for freedom from disease and grown under conditions that precluded infection from outside sources. Also a large number of control plants were grown, thus reducing the chance of error from incipient corm infection.

The inoculations were made by adding the Fusarium cultures to the soil in which the corms were planted. The plants, if susceptible, developed a typical infection, while the adjacent control plants remained healthy. From various parts of the infected plants the *Fusarium* was reisolated and these reisolations, when compared with each other and with the original isolate, were found to agree in cultural and morphological characters. Also, the reisolations were tested by inoculating plants and proved pathogenic.

In a field trial (detailed in the section on varietal susceptibility, p. 284), 18 isolates of the vascular *Fusarium* were tested separately on each of 20 varieties of gladiolus. From the 7 more susceptible varieties reisolations were made from infected vascular tissue of the new corms; these have been compared with the cultures used as inoculum by cultural tests on 5 different media (potato-dextrose, corn-meal, corn-meal-plus-dextrose, and oatmeal agar, and bean pods), and by spore measurements. Judged by these comparisons, the Fusarium isolates recovered from the infected gladiolus are of the same type as those used to inoculate the soil.

Experimental proof was obtained that contractile roots are infected by the vascular *Fusarium* and that they transmit the disease to the new corms. In these tests corms of 2 varieties, Dr. F. E. Bennett and Chas. Dickens, 30 corms of each, were grown singly in sterilized soil in 6-inch pots. The inoculum was applied after the contractile roots were well developed. The surface soil was removed carefully to minimize wounding of roots, a Fusarium culture in a water suspension was poured over these, and the soil replaced. With this method a number of contractile roots became infected and all infection in the new corms could be traced to infected contractile roots. The *Fusarium* was reisolated from a number of contractile roots and corms, and pathogenicity of several of the reisolates was demonstrated. No infections developed in the 10 control plants of each variety.

Observations and experiments indicate that the pathogenicity of isolates from "doughnut" types of infection is less than that of isolates from the typical vascular types. Inoculation with the "doughnut" isolates produced typical vascular infection as often as they did the doughnut type, but infection was slower and less extensive than in parallel inoculations with the vascular isolates.

That pathogenicity of this vascular *Fusarium* is reduced by long periods of culture in artificial media has been generally noted but no extensive records have been kept. Three isolates, Nos. 30, 32, 34, of 1935, tested shortly after isolation, were unusually virulent, causing 100 per cent infection in the varieties Panama, America, and Mrs. Francis King. In the same experiment with the same varieties, four older isolates (1929, 1930, 1933) produced 40, 62, 53, and 68 per cent infections, respectively. In 1936 the isolates of 1935 again gave 100 per cent infection on the varieties Dr. F. E. Bennett, Crimson Glow, and Schwaben. In 1938 the variety Dr. F. E. Bennett was inoculated with No. 34 of the 1935 isolates and No. 14 of the 1929 isolates. Isolate No. 34 caused only moderate infection and isolate No. 14 a mere trace. In 1939 isolate No. 34, used in an experiment for observation of root infection, caused only traces of disease, while younger isolates were definitely virulent. Factors other than age may be partly responsible for such decline in virulence. This change has been observed so frequently that cultures 2 years old or younger have been used in all recent experimental work. In addition to such loss of virulence there are even among recent isolates differences in degree of pathogenicity. Some infect a wide range of varieties of gladiolus, others infect only the most susceptible varieties, and some isolates from typical lesions produce no infections.

CHARACTERISTICS OF THE FUSARIUM

Comparison of Isolates

Beginning in 1923, isolates from the yellows disease have been compared and records made of their characteristics and particularly of their pathogenicity. None of the earlier isolates are now in stock but the records and the camera-lucida drawings of these agree perfectly with those of the past

5 or 6 years. The *Fusarium* cultured from gladiolus of various geographic origins, from many different varieties, and from the several plant parts including old and new corms, fibrous and contractile roots, and leaf bases, have been compared. Also reisolations have been checked against original isolations and single-spore cultures against source cultures. The various isolates are very similar in physiology and morphology, although the character of growth of even a single spore isolate may vary considerably with slight differences in the medium or the environment. Variation in *Fusarium* is an old story and is noted in practically all studies of species of this genus. In 1904 Smith and Swingle (21) wrote of *Fusarium oxysporum:* "Judged by the above descriptions we have had half a dozen species of *Fusarium,* yet all were the product of a single spore."

Most isolates have been similar or identical in appearance on the usual culture media. Sectoring has not been observed in either plate or tube cultures. Differences sometimes occurred that suggested separation of the isolates into several groups, but on repetition of the tests these differences proved inconstant. The isolates from various localities and from different parts of the gladiolus plant have proved, on the whole, strikingly similar in the amount and character of growth and pigment. The only apparently constant cultural distinction noted is a difference in aerial growth in Tochinai (24) agar.[4] On this medium some isolates have a fairly persistent surface growth so dense that the color of the stroma does not show through, while in others the aerial growth usually collapses into a flat, appressed layer much the color of the stroma.

Cultures have shown perhaps more variation in pathogenicity than in cultural character. Of 18 typical, single-spore, young isolates (from pathogenic stock cultures) used to inoculate 10 varieties of gladiolus (known to be susceptible) only 1 (12–1) of the 18 produced infection in all of 10 varieties. Three isolates infected 9 varieties; 9 isolates infected 8 varieties; 3 isolates infected 7 varieties, and 2 isolates infected only 6 varieties.

Comparison with Fusarium oxysporum var. gladioli Massey

In addition to the wilt or yellows-producing *Fusarium* of gladiolus, it is to be noted that *Fusarium oxysporum* var. *gladioli,* described by Massey in 1926 (8), is the cause of a gladiolus disease. A culture of this organism, received from L. M. Massey, sub-cultures and reisolations from it, and isolates made by the writer from typically diseased plants, have been included with the vascular pathogen in parallel tests of cultural and morphological characters and pathogenicity. Five cultures of *F. oxysporum* Schlecht. have also been compared with the two pathogens from gladiolus. Four of these cultures were obtained from Freeman Weiss, the fifth from Miss Lillian Cash. In culture the bulb-rot organism has, in most tests and examinations, shown less abundant aerial growth, less pigment, and wider macrospores than the yellows organism. The mycelium of the former also shows a ten-

[4] Modified Tochinai Agar: Peptone, 0.5 g.; Mono potassium phosphate, 0.5 g.; Magnesium sulphate, 0.5 g.; Maltose, 15.0 g.; Agar, 12.0 g.; Water, 1000.0 cc.

dency to be somewhat coarse and to become woolly with age, while that of the yellows pathogen collapses but remains fine in texture. The most distinctive characteristics of these two Fusaria of gladiolus are the effects on the host.

The yellows Fusarium causes a vascular disease of the growing plant, attacking roots and corms early in their development, seriously infecting and even destroying the plants before maturity. Only in late stages of the disease and under moist conditions is the fleshy tissue of the corm attacked and, even then, it is a question whether this *Fusarium* is chiefly responsible for these late stages of rot, as by this time various secondary organisms have invaded the affected corm.

Fusarium oxysporum var. *gladioli,* on the contrary, attacks the surface and causes a rot of the starchy storage tissues. The lesions originate during the growing period, or perhaps during or after harevsting, and continue to develop during storage. Massey (8) says of *F. oxysporum* var. *gladioli,* "It is primarily a storage disease."

In susceptible varieties of gladiolus grown in soil heavily infested with *Fusarium oxysporum* var. *gladioli,* an occasional slight vascular infection has resulted that would be overlooked on casual examination. Such infections have occurred in several inoculation experiments under conditions that precluded infection from sources other than the inoculum placed in the soil. In parallel inoculations the vascular types of *Fusarium* gave 100 per cent severe infections.

The ability of the 2 organisms to cause exterior lesions on corms was compared by inoculating, with and without needle prick wounds, the surface of 5 varieties of mature corms. The corms were kept slightly moist, at 24° C. for 3 weeks. No infection developed, except through wounds. *Fusarium oxysporum* var. *gladioli,* infected all 5 varieties, producing 46 lesions of moderate size, while the vascular *Fusarium,* with an equal number of wounds, infected only 2 varieties and produced only 10 very small lesions.

Cultural Characters

Cultures have been kept at room temperature (22° to 25° C.) and in moderately subdued light, except in a few special experiments.

In most media there is a fairly abundant mycelial growth, white, fine textured, with little or no pigment, except in media containing sugar or acid. With age the aerial mycelium partly collapses and becomes tinged with buff or cream color. On corn-meal agar, both with and without sugar, and on stems of clover and potato, the aerial growth is scanty. Growth is abundant and strongly pigmented (Spinel-red to dull-purple (19)) on rice. No odor has been detected in rice cultures.

On Tochinai[5] agar, the yellows *Fusarium* makes a vigorous growth and produces abundant and vivid colors. The color begins as pale-pink, changes to red (Spinel-red), or reddish-orange (Carnelian-red), then Vinaceous-

[5] See footnote 4.

purple or Hyssop-violet with numerous intermediate tones and shades of these colors. In well-grown plate and slanted-tube cultures the reverse shows a wide marginal zone of radiating lines of various shades of red, red-orange, or purple. When first used this seemed a useful medium for separation of strains because of the variety of colors produced, but isolates grouped according to color often changed rapidly, requiring regrouping in less than a day. In the end they were all practically alike as to color. As stated (p. 284), the isolates seem to fall into two groups in regard to the character of the aerial growth in Tochinai agar.

Microconidia are abundant in all media; macroconidia absent, few, or irregularly produced; pionnotes, very rare; sclerotia, not infrequent in potato agars, small, dark-gray to dark bluish-green; chlamydospores, abundant in all media. Many kinds of media have been used in the effort to increase the production of macroconidia. Oatmeal hard agar and clover stems have given the best results, but even with these media many cultures failed to produce macroconidia. (Three cultures on clover stem and two on bean stem developed pionnotes or sporodochia.)

Morphologic Characters

From a number of recent single-spore isolates of proved pathogenicity, several (7–3, 11–2, 12–1, and 16–1) were selected in 1940 for the morphological description. These were chosen as representing the slight differences in growth and because they produced a sufficient number of macroconidia for study. They were isolated in 1938 from typically infected vascular tissue of gladiolus. Their pathogenicity was tested and single-spore isolates made in 1939. The pathogenicity of the single-spore isolates was confirmed in 1939 and again in 1940, and the *Fusarium* was again reisolated. In gross and microscopical morphology and in physiology these recent isolates and reisolates are very similar to or identical with other recent isolates and also with the numerous isolates of the past 18 years.

Of the selected isolates, No. 11–2 is representative of the group producing persistent aerial growth and less pigment than the majority of the isolates. Number 16–1 most nearly represents the group with aerial growth that frequently collapses in Tochinai agar. The others are intermediate between these two or variable in this respect. In the latest pathogenicity tests, isolate No. 12–1 ranked highest, with an average of 64.5 per cent infection in 10 varieties of gladiolus; No. 11–2 gave 52.5 per cent infection; No. 7–3, 50.5 per cent, and No. 16–1, 49.5 per cent in the same 10 varieties of gladiolus.

The aerial mycelium is white, abundant, and 3 to 5 mm. deep, with age slightly buff. Sub-stratum, colorless to pale-buff, lilac, Spinel-red, or vinaceous-lilac to dark-vinaceous, depending on the medium.

Sclerotia occur occasionally on potato-dextrose agar. They are small, more or less globose, single or clustered, dark-gray to bluish-green or dark-blue, formed in one or two rows in the lower margin of slant agar cultures.

Chlamydospores develop in all media. They are hyaline; usually vacuolate, spherical and 1-celled (when 2-celled somewhat elongated or pear-shaped); terminal and intercalary

in the mycelium and in the macrospores; mostly single but sometimes in chains of three. The walls are smooth (rarely in very old cultures somewhat irregular or rough), mostly thin, becoming thicker with age. The 1-celled are 7 to 10 μ in diameter, the 2-celled 12 to 15 μ.

Microconidia are abundant in all isolates: hyaline; continuous or 1-septate; 90 per cent ovoid, a few kidney-shaped or ovate (Fig. 4). The size ranges from 3 to 14 × 2.2 to 5.0 μ, but the majority are 7 to 10 × 2.8 to 4.2 μ.

Macroconidia are scarce, often lacking. In one culture tube (not a single cell isolate) a few *sporodochia* were found. *Pionnotes* are rare and, as stated earlier, have been observed in only three culture tubes (clover stem, bean stem, corn-meal agar). These three were the only cultures found with a large number of macroconidia. In most cases the macroconidia are scattered in the aerial mycelium along with the microconidia. Even when macroconidia were reasonably numerous, their size, especially the diameter and the septation, varied not only in the different media, but also at different ages in the same medium. As an example, parallel cultures of isolate No. 16–1 were made in oatmeal agar, Tochinai agar, potato-dextrose agar, corn-meal agar, and rice. In oatmeal and Tochinai agars the macrospores were 3.5 to 5.6 μ in diameter, while at the same age (24 days) the spores from rice, corn-meal, and potato-dextrose agar were only 3.0 to 4.5 μ in diameter. Potato-dextrose-agar cultures 10 days old had macrospores 3.0 to 4.4 μ in diameter. Ten days later, spores from the same tube were 4.2 to 4.9 μ in diameter.

The macroconidia seem not to remain long in what is considered a normal condition. If too young they do not have the width of mature spores and the septation is poorly developed. When too old the spores are either shriveled or swollen (depending on the moisture content of the medium), hyaline, full of vacuoles, often with broken or absorbed apical cells, or with chlamydospores in the cells or at the ends. Such changes have been noted in all of the isolates. If macroconidia are produced at all they reach a stage of normal development and then rather rapidly deteriorate, even disintegrate. The normal, mature macroconidia in good condition are homogeneous or finely granular, with walls and septa well developed, and cells neither swollen nor shrunken. They are mostly straight, yet many are slightly curved. The central part is nearly straight and of equal diameter; the ends are pointed, the base with a pedicel more or less developed, and the apex curved or bent (Fig. 4).

Because of the scarcity of macroconidia it was decided to try to find and measure 50 of the 3-septate spores from as many isolates as possible and all spores with other septation found during the search for the 50. In many cases it was necessary to search a number of mounts to secure the desired number. In those unusual mounts in which macroconidia were relatively numerous, 100 of the 3-septate forms were measured. The 4- and 5-septate spores are rare and only one 6-septate spore has been seen.

For measuring, the spores were mounted in water on a thin layer of clear agar and the cover sealed with vaseline. The slides were prepared by flooding them with melted water agar, drained quickly, and dried. When the spores, in a drop of water, are mounted on such a slide the agar film softens sufficiently to hold the spores in place and in one plane. The spores were measured immediately after mounting. Such slides if protected from dust remain in good condition for days.

An attempt was made to have the spores to be measured of the same age, grown in the same medium and under the same conditions. This was not entirely successful, as the development of spores was variable, even under

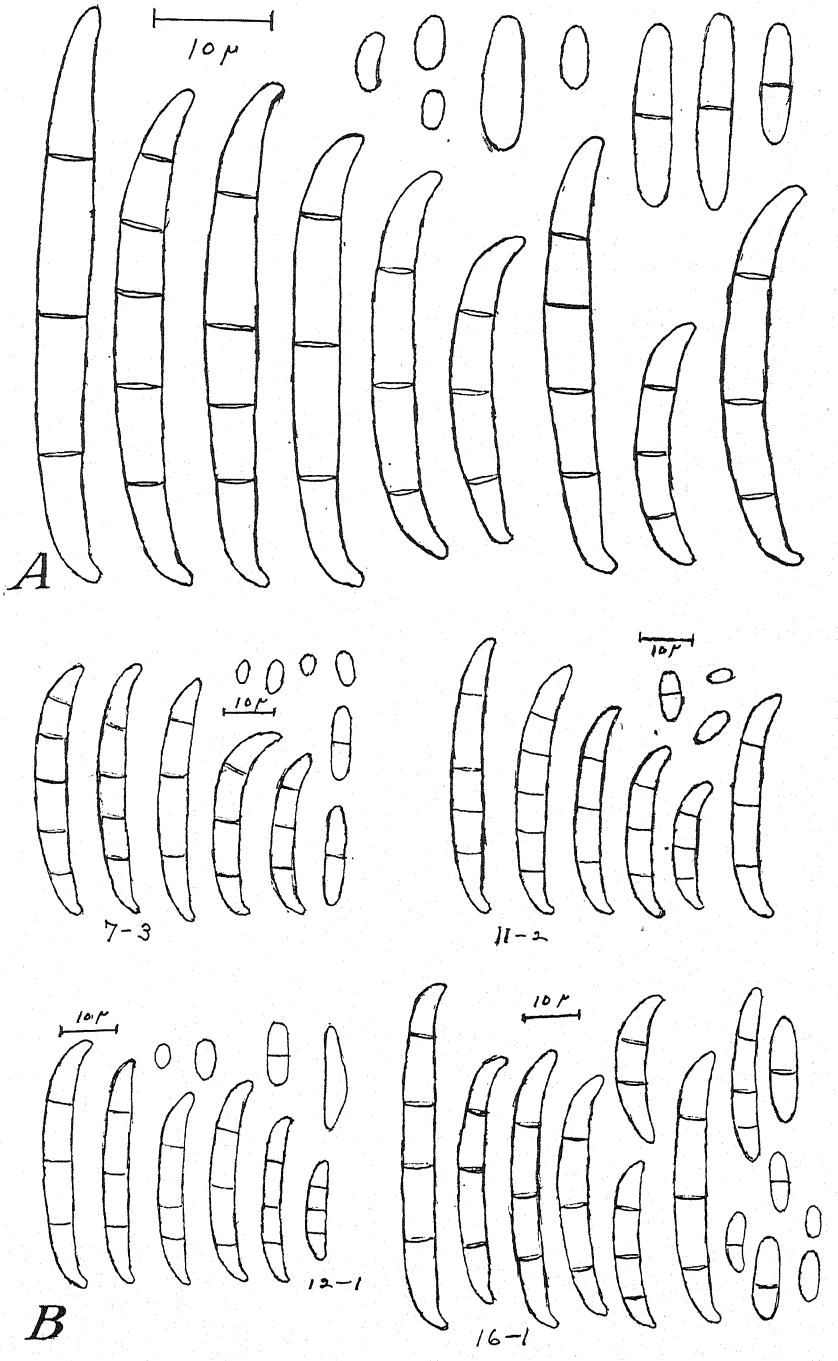


FIG. 4. *A*. Spores of *Fusarium orthoceras* var. *gladioli* from potato-dextrose-agar culture. *B*. Spores of *F. orthoceras* var. *gladioli*, produced by single-spore cultures of isolates 7–3, 11–2, 12–1, and 16–1, respectively. (See table 1 for media.)

apparently identical conditions. In some cultures, normal macroconidia were present in 14 to 18 days, in others, not until considerably later. Isolate No. 7–3, when 20 to 30 days old, had normal 3-septate macroconidia, and 6 to 9 per cent of 4- and 5-septate spores. When 45 days old, the 3-septate spores were scarce and in poor condition and none of the 4-septate or 5-septate type were found. Just the reverse occurred with No. 7–3B in the same medium, where the 3-, 4-, and 5-septate spores were normal and more numerous at 45 days.

Measurements of macroconidia from 4 representative single-spore isolates are given in table 1.

Regardless of some variations in morphology, physiology, and degree of pathogenicity, these isolates from gladiolus are closely similar and have enough stable characters to indicate that they belong to one species in the sub-section Orthoceras. It seems legitimate to establish this *Fusarium* as a new variety on its known host relationship. The apparent restriction to gladiolus (including probably some related plants, as is indicated in the section entitled Other Potential Hosts), seems sufficient reason for describing it as a new variety.

Classification

Because of its morphological and physiological characters it is fairly easy to determine that this vascular pathogen of gladiolus belongs, according to the classification of Wollenweber (26) and Wollenweber and Reinking (27), in the section Elegans. It is somewhat more difficult to decide between the sub-sections Orthoceras and Oxysporum. The rare production of pionnotes, the few sclerotia, an occasional culture with numerous macroconidia, and the fact that *Fusarium oxysporum* var. *gladioli* is able to cause a slight vascular infection in gladiolus, suggested a relationship with the sub-section Oxysporum and possible identity with *F. oxysporum* var. *gladioli*. But these similarities are not sufficient to balance the entirely different disease symptoms produced in the host by the vascular pathogen, the general nonproduction of sporodochia, pionnotes, or sclerotia, and the more slender and straighter macroconidia, all of which indicate a closer relationship to the Orthoceras than to the Oxysporum sub-section.

There is not sufficient discrepancy in the physiological or morphological characters of the four selected representatives or among the hundreds of other isolates from gladiolus to warrant further separation within the variety. In certain respects the variations found are considered no greater than those occurring in many if not most species and varieties of *Fusarium*.

An important distinction of the vascular parasite of gladiolus is its host relationship, which seems sufficiently important to establish it as a variety of the type species of the sub-section. The name *Fusarium orthoceras* var. **gladioli** n. var. is proposed for this pathogen.

Technical Description

Fusarium orthoceras App. et Wr. var. **gladioli** new var.

Mycelium typically aerial, white, abundant, fine-textured; on corn-meal agar appressed, colorless. Color of substratum in beef-agar cultures buff to chamois; in oatmeal

TABLE 1.—*The range and average size in microns of the macroconidia of 4 representative cultures of Fusarium orthoceras var. gladioli isolated from gladiolus in 1938. Single-spore isolates in 1939. Measurements made 1939–40*

Isolate number	Source of isolate	Age of culture	Macroconidia measured		Measurements	
					Range	Average
			Septations	*Number*	*Microns*	*Microns*
7–3	Husk tissue of stored corm (variety Blue Boy) grown in Michigan 1937.	30 days on oatmeal agar	0	0	.....	.....
			1	9	15.5–19.6 × 3.5–4.2	.....
			2	2	16.8–19.6 × 3.5	.....
			3	100	16.8–47.6 × 3.0–4.8	31.8 × 4.2
			4	9	30.0–45.5 × 4.2–4.5	.....
			5	6	36.4–40.6 × 4.2–4.5	.....
			6	0	.....	.....
11–2	Vascular tissue of new corm (variety Mrs. Frank Pendleton) grown in soil inoculated with vascular *Fusarium*, isolate 11.	34 days on oatmeal agar	0	0	.....	.....
			1	2	14.0–15.5 × 3.5	.....
			2	1	19.6 × 4.0	.....
			3	50	16.8–49.0 × 2.8–5.6	30.8 × 4.2
			4	2	32.2–36.5 × 3.5–4.2	.....
			5	1	25.5 × 4.3	.....
			6	0	.....	.....
12–1	Vascular tissue of new corm (variety Mrs. Francis King) grown in soil inoculated with vascular *Fusarium* 12.	35 days on potato dextrose agar	0	0	.....	.....
			1	3	18.0–22.0 × 3.0–4.4	.....
			2	1	19.6 × 4.2	.....
			3	50	18.2–37.8 × 3.5–4.3	28.3 × 3.8
			4	0	.....	.....
			5	0	.....	.....
			6	0	.....	.....
16–1	Vascular tissue of new corm (variety Mrs. Francis King) grown in soil inoculated with the vascular *Fusarium* 16.	24 days on oatmeal agar	0	0	.....	.....
			1	5	25.0–25.5 × 4.2	.....
			2	0	.....	.....
			3	100	22.5–54.6 × 3.5–5.6	35.7 × 4.3
			4	1	53.2 × 4.4	.....
			5	0	.....	.....
			6	0	.....	.....

agar pale-pink to Spinel-red and vinaceous-purple;[6] in rice, aerial mycelium white, submerged hyphae and rice pale-pink to Spinel-red, Jasper-red and dark, dull-purple; no odor from rice cultures. Microconidia abundant, mostly non-septate, 90 per cent ovoid, 3–14 × 2.2–5 μ, mostly 7–10 × 2.8–4.2 μ; the few 1-septate, 14–2.5 × 3.0–5 μ. Macroconidia typically scarce; scattered in the aerial mycelium, very rarely in pionnotes; straight to slightly curved; slightly pedicellate, moderately dorsiventral; ends pointed and curved; typically 3-septate, 31.5 × 4.2 range (16.8–54.6 × 2.5–5.6); 4-septate, rare, 40.9 × 4.7 (range 30.0–53.0 × 3.5–5.6); 5-septate, rare, 41.5 × 4.7 (range 25.5–54 × 4.2–5.6); the 0-, 1-, and 2-septate macroconidia, rare in mature cultures, 10–40 × 2.5–4.5, average diameter 3.6. Sporodochia none; pionnotes very rare; sclerotia occasional. Chlamydospores abundant in mycelium and macroconidia; terminal and intercalary; spherical to oval; single and in short chains; smooth; single, 7–10 μ, 2-celled, 12–15 μ. Optimum temperature 23° to 26° C. Parasitic in tracheae of fibrous and contractile roots and corms of gladiolus; cause of Fusarium yellows in horticultural varieties of gladiolus, of the Nanus, Primulinus, and Grandiflorus forms.

TEMPERATURE RELATIONS

Effect of Temperature on Growth of Fusarium in Culture

In slanted tubes of potato-dextrose agar sealed to prevent evaporation, the minimum temperature for growth of the vascular *Fusarium* is below 3° C., a trace of growth becomes visible in 4 days at 3° C. The optimum lies in the range of 23 to 26° C. and the maximum at about 34 to 36° C. At 34° C., growth visible with a hand lens increases slowly but remains scanty and restricted to the inoculated area. No apparent growth occurs in 2 weeks at 36 and 37° C., but when the tubes are removed to 24° C., normal and abundant growth develops in 5 days.

Freshly inoculated potato-dextrose tubes kept at −20° C. for 35 days, then removed to 24° C., developed visible growth in 3 days and the slants were covered by normal growth in 6 days.

The effect of soil temperature was determined by growing gladiolus in metal cans suspended in Wisconsin type tanks of water. The variation in the water temperature, maintained and controlled electrically, was slight, not more than 1 to 2° C. The temperatures ranged from 15 to 32° C. A suitable moisture content of the steam-sterilized soil was kept constant. Cultures of the *Fusarium* were added to the soil below the corms. To retard evaporation, peat or granulated cork was placed on the soil surface.

Infection of roots and parent corms occurred throughout the range of soil temperatures to which these structures were exposed. Plant growth was best at 15 to 18° C., and, at these temperatures, the new corms often escaped infection. Both the number of plants infected and the degree of infection increased up to 22 to 25° C. Soil temperatures above 25° C. are evidently unfavorable for both the gladiolus and the pathogen, as infections were practically limited to the roots and the base of parent corms.

Three experiments in 3 different years, including 7 susceptible varieties of gladiolus, indicate that the optimum soil temperature for infection is 22 to 25° C. (Table 2). For the records in this table, every trace of infection, regardless of its extent, was regarded as positive. This does not give a true picture as 100 per cent infection at 15° or 18° C. usually represented a much slighter degree of infection than 100 per cent at 22° to 25° C.

[6] Plate 44 of Ridgway's Color Chart. There is another vinaceous purple on plate 38.

TABLE 2.—*Effect of soil temperature on incidence of vascular disease in gladiolus grown in soil inoculated with Fusarium orthoceras var. gladioli, F. oxysporum var. gladioli, or Trichoderma sp.*

Soil temp.	1932 experiment[a] Infection in parent corms and new corms inoculated with					
	F. orthoceras var. *gladioli*		*F. oxysporum* var. *gladioli*		Uninoculated controls	
	Parent corms	New corms	Parent corms	New corms	Parent corms	New corms
°*C.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
15	75	38	0	0	0	0
18	60	25	0	0	0	0
21	45	50	0	0	0	0
24	44	70	0	0	0	0
28	43	(*)	0	(*)	0	(*)
32	15	(*)	0	(*)	0	(*)

	1934 experiment[b] Infection in new corms inoculated with					
	F. orthoceras var. *gladioli* Isolate			*F. oxysporum* var. *gladioli*	*Trichoderma* sp.	Uninoculated controls
	#1	#2	#3			
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
15–16	66.6	83.0	0	0	0	0
18–19	81.2	12.5	0	0	0	0
24	100.0	50.0	50.0	0	0	0
28	(*)	(*)	(*)	(*)	(*)	(*)

	1936 experiment[c] Infection in new corms inoculated with *F. orthoceras* var. *gladioli*							
	Isolate							Uninoculated controls
	#13	#14	#15	#23	#30	#32	#34	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
16	50	25	0	50	75	75	75	0
19–20	0	0	25	0	100	100	50	0
26–27	0	25	0	75	50	75	100	0

(*) No new corms developed.

[a] Test varieties, Mrs. Francis King, Schwaben, Tyrian Beauty.

[b] Test varieties, Mrs. Francis King, Mrs. Frank Pendleton, and one unknown variety.

[c] Test varieties, Dr. F. E. Bennett, Schwaben, Crimson Glow.

In experiment 3 all of the parent corms in the inoculated lot were infected. In contrast all of the parent corms in the control lot remained free from disease.

In addition to the noninoculated controls in each experiment, *Fusarium oxysporum* var. *gladioli* and *Trichoderma* sp. were used to inoculate the soil and were carried at the same temperatures as the vascular *Fusarium*. No infections resulted from these, and the plants developed normally and exactly like the controls.

VARIETAL SUSCEPTIBILITY

Among the comparatively few varieties of gladiolus used in experiments, differences in reaction to the vascular parasite ranged from high resistance to complete susceptibility. In parallel experiments in heavily infested soil and with conditions favorable for the parasite, the susceptible varieties developed extensive infection and died before blooming. The highly resistant varieties were only occasionally and slightly infected (2 to 4 per cent) and usually without any foliage symptoms, the infection being discovered only by cutting the corms.

Both field and greenhouse tests have demonstrated (12) that the following varieties of gladiolus can be listed as resistant: Albania, Alice Tiplady, Apricot Glow, Dearborn, Giant Nymph, Hopi, Los Angeles, Minuet, W. H. Phipps, Picardy, Souvenir, and Spirit of St. Louis.

The following varieties have been found susceptible: America, Anna Eberius, Ave Maria, Bloomington Beauty, Blue Boy, Byron L. Smith, Charles Dickens*, Chicago White, Commander Koehl, Crimson Glow*, Dr. F. E. Bennett*, Emile Aubrun, Edith Mason, Evelyn Kirtland*, Flaming Sword, Golden Measure*, Halley, Herada, Kalamazoo, Lansing, Le Marechal Foch, Maidens Blush*, Marie Kunderd, Mary Shary, Mrs. F. L. Karcher*, Mrs. Francis King*, Mrs. Frank Pendleton, Niagara, Odin, Orange Queen, Panama, Pride of Goshen, Pride of Wanakah, Purple Glory, 1910 Rose, Schwaben, E. J. Shaylor*, Tempa, Tyrian Beauty, Virginia*, War*. Varieties starred above have proved very susceptible in our tests; leaf yellowing and wilt occur almost as soon as the leaves appear and the whole plant is usually dead before flower buds form. The other varieties show foliage symptoms later in the season and the new corms may escape infection if growing conditions are favorable.

It is interesting to note that the resistant variety Picardy is the off-spring of a cross between Apricot Glow, a resistant variety, and Emile Aubrun, which is of intermediate rank in the writer's experiments. It, therefore, seems possible to secure resistance in a new variety even when one parent is more or less susceptible.

Other Potential Hosts

In parallel inoculation experiments on growing gladiolus, freesia (*Freesia* sp.), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), and celery (*Apium graveolens* L.), typical vascular infections were produced in the gladiolus, but no trace of infection of any sort in potato, tomato, or celery, and no definite infection in the freesias. Inoculation tests on other hosts might add to the list of host plants. At present, the

writer has found this *Fusarium* in *Gladiolus, Tigridia,* and *Montbretia* (*Tritonia*), but has proved its pathogenicity only to gladiolus.

In 1923 freesia corms from California showed disease symptoms similar to those of the vascular disease of gladiolus, and, when planted, behaved exactly as the infected gladiolus. The *Fusarium* isolated from the freesias appeared identical in morphological and cultural characters with the gladiolus *Fusarium* but cross-inoculation experiments made in 1923–24 were not successful. Unfortunately, the variety of gladiolus used in these experiments was not recorded. The freesias used were of the variety Purity. It could be that the failure to infect was due to resistant varieties. Taubenhaus and Ezekiel (22, 23) have described a Fusarium wilt and corm rot of freesias. They failed to secure typical infection of gladiolus on inoculation with the *Fusarium* from freesia. Here again the gladiolus used may have been resistant.

In 1927 the writer isolated from Montbretia and Tigridia corms showing brown vascular rot, a *Fusarium* morphologically and physiologically like that from gladiolus. This fungus proved pathogenic to gladiolus, producing the same vascular type of infection as the *Fusarium* isolated from gladiolus. As test plants were not available, no pathogenicity tests were made on *Montbretia* or *Tigridia.* Crocus corms (*Crocus* sp.) with typical vascular infection have been examined by the writer, but no isolations were made. Drayton (5) has reported a vascular Fusarium disease of crocus.

CONTROL

It is difficult to control a parasite that penetrates so deeply into plant tissues that it cannot be reached by disinfectants or destroyed by temperatures not injurious to the host. Prolonged high or low temperatures, with or without moisture in the soil or in cultures, have had little or no effect in reducing the vitality of the parasite. Hot water treatments, employing various combinations of temperature and time of exposure were useless because the parasite proved more resistant than the host.

Numerous combinations of soil types with soil temperature, soil moisture, soil pH values, and fertilizers have been tried without success in securing clean crops from infested soil or infected planting stock. As a soil disinfectant, chloropicrin (10) was effective in destroying the *Fusarium.* In the chloropicrin-treated plots, even the susceptible gladiolus varieties grew to maturity in perfect health, while the same varieties in adjoining, untreated plots, developed infection and died before blooming.

Infected corms should be disposed of in a manner to prevent spreading the disease. Planting stock should be disinfected to destroy surface organisms. A 5-minute dip in mercurous chloride (Hg_2Cl_2) 3 to 5 ounces in 1 gallon of water is the most efficient disinfectant tried by the writer. Mercuric chloride ($HgCl_2$) 1–1000 for 6 to 8 hours is very efficient. With corms of the "doughnut" type of infection, experiments indicate that disinfection before planting results in a degree of control not attained in the usual vascular type.

As this parasite is now widely distributed and persistent in the soil and soil disinfection is too costly for large-scale use, the selection and use of resistant varieties of gladiolus appear to be the surest method of avoiding the disease. A number of resistant varieties are available (see list, p. 284) covering a wide range of colors and forms. By concentrating on these for breeding, a larger number of resistant varieties should be obtained.

SUMMARY

A disease of gladiolus, widespread and destructive in the United States, is caused by a *Fusarium* that attacks primarily the vascular tissues of the plant. It causes premature yellowing and death of the leaves, and eventual destruction of the corm.

This disease was first observed by the writer in 1923, in corms grown on the Pacific Coast from stock originally imported from Europe. From the West the disease seems to have spread rather rapidly eastward, probably largely by distribution of infected planting stock that, in external appearance, was free from disease.

The fungus enters the roots and the base of the corm and advances upward through the core, browning and destroying the vascular tissues. Many badly infected corms have no exterior sign of disease as only in the late stages of infection are discoloration and rot apparent on the surface of the corm.

The source of the infection may be either infected planting stock or infested soil. Infected planting stock produces poor growth and a poor crop or none; the disease is transmitted directly from the infected parent corms to any new corms that may develop. Healthy corms planted in infested soil become infected chiefly through the roots, both fibrous and contractile, or more rarely, through the leaf bases.

Numerous isolations and inoculation experiments have proved that a *Fusarium* is the cause of the vascular rot. The observed variations in cultural and morphological characters of the numerous isolates seem insufficient to warrant their separation into varieties or forms, since all have in common the most important character, the ability to cause a specific disease in the gladiolus.

The essential characters of this *Fusarium* place it, according to the classification of Wollenweber and Reinking, in the section Elegans and sub-section Orthoceras. To avoid establishing a new species it is described as a variety of the type species: *Fusarium orthoceras* var. **gladioli** n. var.

The optimum temperature for growth of the *Fusarium* in culture is 23° to 26° C. In soil artificially inoculated with the *Fusarium*, corm infection occurred at all the temperatures tried (15° to 32° C.). The optimum soil temperature for typical and extensive infection of corms is 22° to 25° C.

Not all varieties of gladiolus are susceptible to this disease. On the basis of the writer's tests, 12 varieties are listed as resistant, 30 as moderately susceptible, and 11 as very susceptible. Experimental infection has been

proved in gladiolus only, but a *Fusarium* very similar to the gladiolus pathogen has been isolated from corms of *Montbretia* and *Tigridia*.

The *Fusarium* can be eradicated from soil with chloropicrin but at present this is expensive for large fields. Selection of clean corms, fungicidal dips for planting stock, and planting in uncontaminated soil, are suggested. The most promising means of control is the selection and use of resistant varieties.

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QUANTITATIVE STUDIES WITH CARBORUNDUM AND ITS USE IN LOCAL-LESION TESTS

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INTRODUCTION

The discovery that leaves of various host plants, when rubbed with a solution of tobacco-mosaic virus, develop necrotic lesions at the point of entrance of infective units into the cells has afforded to plant virus research one of its most useful techniques (3). Several improvements on the original technique that were mostly directed towards the elimination of certain experimental errors have been made. Today the technique of estimating virus concentration by local-lesion tests is widely used by plant virologists. It is well known that the study of any virus is greatly facilitated if a test plant giving satisfactory local lesions is available. More than a dozen plant viruses are known to produce local lesions suitable for measuring virus activity (5).

It is generally accepted that, when the leaf surface of a host is rubbed with a virus suspension, the infective units gain entrance into wounded cells of the epidermis and the trichomes. However, only a relatively small number of cells in the surface of the leaf will be wounded in the proper manner to support lesion development. Without considering the susceptibility of the host, the number of lesions obtained in the inoculated leaves is influenced by two main factors: the number of infective units present in the inoculum and the number of cells that are wounded in the right manner. It is clear that the higher the number of infective units present in the inoculum the greater the chance that some will get into properly injured cells.

It has been pointed out (12) that only a small fraction of the infective units of an inoculum succeed in causing lesions. Most of them are lost on the surface of the leaf, in the inoculating pad, etc. It is therefore clear that the number of lesions will increase as the number of appropriate entry points is increased. While the virus concentration may be readily controlled within certain limits, it is not so easy to control the number of entry points. However, it is possible to increase the number of appropriate entry points by the use of abrasives.

The first abrasive used for plant virus inoculation was sand (2, 10). It was found later (8, 9) that carborundum was of great value as an abrasive, and today its use is widespread for the transmission of viruses difficult to pass mechanically. The carborundum or other abrasive particles are believed to make small openings in the epidermal cells or hairs that afford an entrance for the infective units without killing the cells or wounding them severely (9).

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It is surprising that, in spite of a few good results obtained with abrasives in the inoculation of viruses causing local lesions (9, 10), carborundum has been little used in determinations of virus activity. Its use has been restricted almost entirely to the mere transmission of virus diseases. Black (1), working with potato yellow-dwarf virus, seems to have been the first to use carborundum regularly in local-lesion tests.

It is the purpose of this paper to show that, by using carborundum in local-lesion tests, a manifold increase in the number of lesions is obtained on certain host plants with different viruses. The use of carborundum renders the test more sensitive, and this permits estimation of the concentration of virus preparations that could not be measured satisfactorily otherwise.

MATERIALS AND METHODS

Some of the tests herein reported were carried out at the Department of Horticulture, University of Wisconsin. Others were performed at the Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N. J.

Viruses Used

Three viruses were used in the tests: ordinary tobacco-mosaic virus (*Marmor tabaci* H. var. *vulgare* H.), cucumber-mosaic virus (*M. cucumeris* H. var. *vulgare* H.), and severe-etch virus (*M. erodens* H. var. *severum* H.).

The inocula were obtained by crushing leaves of diseased plants in a mortar and straining through cheesecloth. The plant extract was then centrifuged.

Test Plants

Plants of *Nicotiana glutinosa* L., of the hybrid *N. tabacum* L. × *N. glutinosa* (6), and of *Phaseolus vulgaris* L. var. Early Golden Cluster were used for tests with ordinary tobacco-mosaic virus. *Vigna sinensis* (L.) Endl. var. Black was used exclusively for cucumber-mosaic virus. *Physalis peruviana* L. was used for the inoculations with severe-etch virus (5).

On the tobacco hybrid and *Nicotiana glutinosa,* 5 or 6 consecutive leaves, as uniform as possible, were used per plant, the other leaves and the top being removed before inoculation. *Phaseolus* and *Vigna* plants were inoculated on the first pair of leaves that developed above the cotyledons when they were almost three-fourths of their full size. This generally was 10 days after sowing. Plants of *Physalis peruviana* were used when they had about 6 good-sized leaves.

Inoculation

Inoculations, with the exception of those on *Vigna,* were made by the half-leaf method (10). The carborundum treatment and control were inoculated on the two halves of the same leaf. Inoculations were made in such a way that each treatment or control would occur the same number of times on the right and left halves. The position on the plant also was

TABLE 1.—*Influence of carborundum on the number of local lesions produced on the hybrid, Nicotiana tabacum × N. glutinosa by tobacco-mosaic virus diluted to various concentrations*

Experiment No.	Dilution,[a] treatment, and average number of lesions[b]									
	10^{-4}		10^{-5}		10^{-6}		10^{-7}		10^{-8}	
	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
1	288.6	11.6	30.5	0.6	9.3	0.7	.....	.....	.....	.....
2	262.7	5.2	38.2	1.0	20.8	2.6	.....	.....	.....	.....
3	215.7	15.1	18.8	2.1	7.2	0.5	5.2	0.2	.....	.....
4	174.7	19.2	39.4	5.3	13.1	0.0	1.6	0.0	0.8	0.0
5	378.1	43.4	72.4	5.9	17.6	1.1	1.6	0.0	2.5	0.0
Average	264.0	18.9	39.9	3.0	13.6	1.0	2.8	0.1	1.7	0.0

[a] Diluent distilled water.
[b] Average number of lesions per half-leaf on 10 half-leaves.

TABLE 2.—*Influence of carborundum on the number of local lesions produced on Nicotiana glutinosa by tobacco-mosaic virus diluted to various concentrations*

Experiment No.	Dilution,[a] treatment, and average number of lesions									
	10^{-4}		10^{-5}		10^{-6}		10^{-7}		10^{-8}	
	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
1[b]	310.8	10.7	82.8	2.5	11.0	0.3	3.2	0.1	1.5	0.1
2[b]	400.0	8.7	113.3	2.7	32.2	0.5	26.1	0.1	10.3	0.0
3[b]	326.3	16.3	136.1	2.6	31.3	0.3	11.0	0.1	1.7	0.0
4[b]	196.6	3.7	17.2	0.3	4.7	0.0	1.4	0.2	0.6	0.1
5[c]	146.4	2.6	30.4	0.6	2.2	0.0	0.2	0.0	0.0	0.0
6[d]	278.0	8.8	71.1	1.1	6.0	0.1	0.7	0.0	0.5	0.2
Average	276.4	8.5	75.2	1.6	14.6	0.2	7.1	0.1	2.4	0.1

[a] Diluent distilled water.
[b] Average number of lesions per half-leaf on 12 half-leaves.
[c] Average number of lesions per half-leaf on 18 half-leaves.
[d] Average number of lesions per half-leaf on 15 half-leaves.

changed so as to allow the various treatments and controls to occur the same number of times at the different leaf positions. With *Vigna sinensis* the carborundum treatment and control were inoculated always on opposite leaves of the same plant.

Two grades of carborundum were used for the majority of tests: a 600-mesh type manufactured by Braun-Knecht-Heimann Company, San Francisco, California, and a 320-mesh type of silicon carbide manufactured by the Carborundum Company, Niagara Falls, N. Y. Carborundum was applied in different ways: by dusting with a small duster, by sprinkling from a vial with a perforated lid or a vial with cheesecloth around the mouth, and by mixing directly in the inoculum. In general, carborundum was applied after the control inoculations had been performed.

The inoculations were made with a small folded piece of cheesecloth dipped into the inoculum and then gently rubbed on the entire surface of the leaf or half-leaf. Usually 6 strokes were used, but this number was increased in the case of the tobacco hybrid to 10 or 12 strokes. Shortly after inoculation the leaves were rinsed with water. Lesions were usually counted 96 to 120 hours after inoculation except in the case of those produced in *Physalis peruviana*. The lesions obtained on this plant were counted 10 days after inoculation.

EXPERIMENTAL

Effect of Carborundum on the Number of Local Lesions Caused by Ordinary Tobacco-mosaic Virus on the Tobacco Hybrid, Nicotiana glutinosa, and Phaseolus vulgaris

Several trials were performed with these test plants using solutions of tobacco-mosaic virus at various concentrations in water or 0.1 M neutral phosphate buffer. In all experiments performed with the tobacco hybrid, 600-mesh carborundum was used, and with *Nicotiana glutinosa* and *Phaseolus* the 320-mesh type. The results of the experiments performed are presented in tables 1 to 3.

Carborundum increased greatly the number of lesions obtained in these 3 test plants, as may readily be seen from the tables. The increase was greater on *Nicotiana glutinosa* and *Phaseolus* than on the tobacco hybrid. This discrepancy may, however, be due to the difference in the 2 types of carborundum or to other differences, resulting from the fact that the experiments were performed at different places.

The increase in number of lesions over the controls, brought about by the use of carborundum, seemed to vary somewhat at different dilution levels. However, the data are not extensive enough to permit a good comparison of the dilution curves with and without carborundum. It can be said that the curves are approximately parallel except at high dilutions. Figure 1 shows the curves obtained with and without carborundum on the 3 host plants.

In some experiments the use of carborundum increased the variation. This was noticeable only when comparisons were made between samples giv-

ing approximately the same number of lesions per half-leaf. Since the variation tended to increase as the numbers of lesions decreased and since carborundum caused an increase in number of lesions, the controls in general

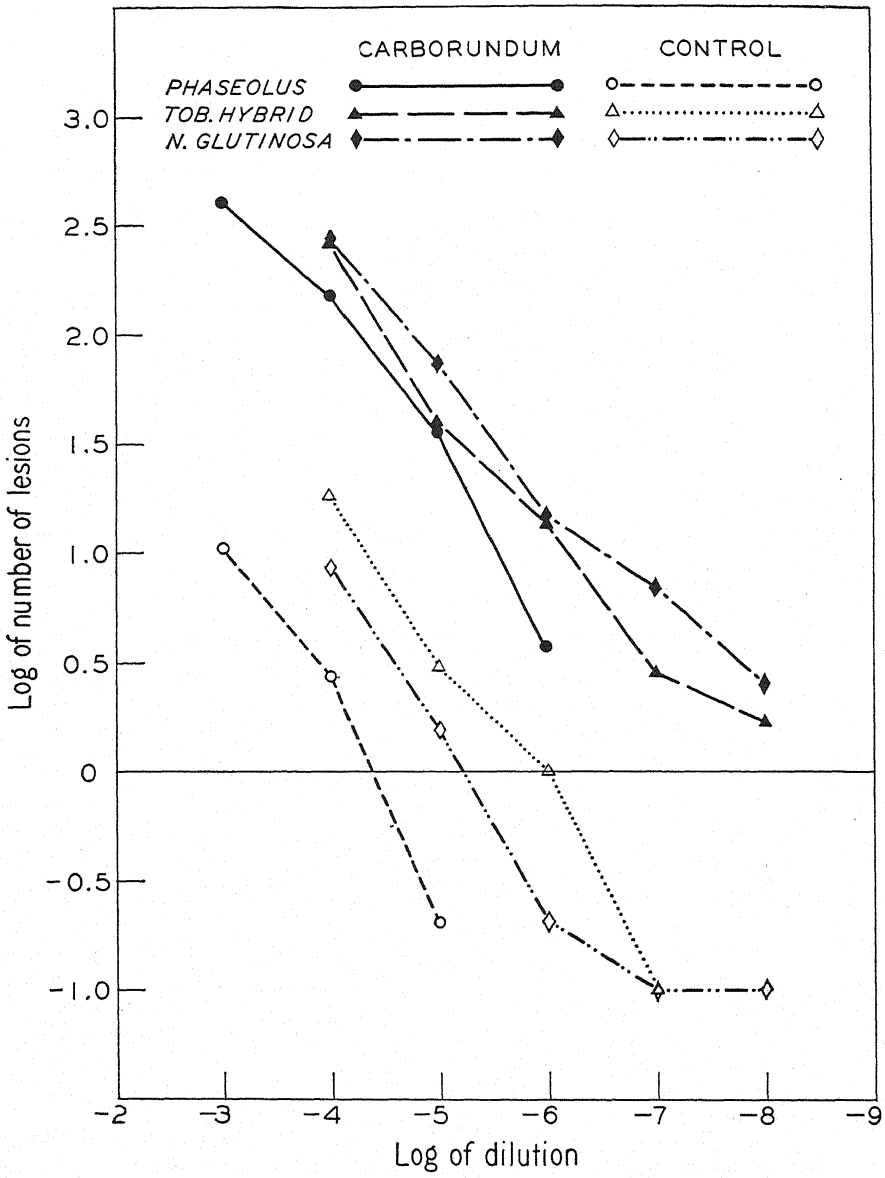


FIG. 1. Dilution curves of tobacco-mosaic virus when inoculated with and without carborundum on *Phaseolus vulgaris* var. Early Golden Cluster, *Nicotiana tabacum* × *N. glutinosa*, and *N. glutinosa*.

showed a greater variation when comparisons were made between samples of the same virus concentration.

With the tobacco-hybrid test plants the increase due to carborundum was more noticeable in the top leaves, the number of lesions being 8.8, 9.2,

TABLE 3.—*Effect of carborundum on the number of local lesions caused by tobacco-mosaic virus on Phaseolus vulgaris var. Early Golden Cluster*

Experiment No.	Dilution,[a] treatment, and average number of lesions[b]							
	10^{-3}		10^{-4}		10^{-5}		10^{-6}	
	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
1	460.0[c]	14.4	196.3	4.8	53.6	0.3	6.4	0.0
2	370.0[c]	9.1	124.6	1.2	28.4	0.3	1.7	0.0
3	416.7[c]	7.2	130.6	2.1	24.2	0.1	3.1	0.0
Average	415.6	10.2	150.5	2.7	35.4	0.2	3.7	0.0

[a] Diluent 0.1 M neutral phosphate buffer.
[b] Average number of lesions per half-leaf on 30 half-leaves.
[c] Number of lesions was estimated.

13.6, 19.8, and 33.8 times the number obtained with the control preparations for the 5 leaves, counting from the bottom. On *Nicotiana glutinosa* the increase in number was, at the different leaf positions counting from the bottom, 35.0, 57.4, 59.3, 50.5, 45.0, and 31.1 times those of the control preparations at the same positions.

The Action of Carborundum Is on the Test Plant. Some experiments were made to find out whether carborundum had any action on the virus. A solution of tobacco-mosaic virus at 10^{-4} in distilled water was divided into 3 portions. To 2 portions 600-mesh carborundum was added. After this had been done, the mixture of virus and corborundum was shaken for a while and then the carborundum was filtered out of one of the portions with ordinary filter paper. The 3 preparations—control, virus solution with carborundum, and virus solution to which carborundum had been added and removed—were compared on the leaves of the tobacco hybrid test plant. The results presented in table 4 show that carborundum had no effect on the virus. The preparation to which carborundum had been added and removed gave nearly the same number of lesions as the control.

Methods of Applying Carborundum. A few experiments were carried out to try 3 different methods of applying carborundum: sprinkling or dusting the carborundum on the leaves of the test plant or adding it directly to

TABLE 4.—*Influence of carborundum on the number of local lesions caused by tobacco-mosaic virus on the hybrid, Nicotiana tabacum × N. glutinosa. Inoculum diluted 1: 10,000*

Experiment No.	Treatment and average number of lesions[a]		
	Carborundum added to inoculum	Carborundum added, then filtered out	Control
1	269.9	22.6	25.0
2	210.3	20.8	15.6
3	326.2	33.6	29.1
Average	268.8	25.7	23.2

[a] Average number of lesions per half-leaf on 10 half-leaves.

the inoculum. The results of the trials presented in table 5 show that there were no significant differences among the methods of applying 600-mesh carborundum as tested on the tobacco hybrid test plant.

Comparative Effect of Different Grades of Two Abrasives. These experiments were performed with 5 grades of 2 types of abrasives, carborundum (silicon carbide) and Aloxite (aluminum oxide). Both powders are manufactured by the Carborundum Company.

The inoculum used in these experiments was a solution of tobacco-mosaic virus at $\frac{1}{5} \times 10^{-4}$ in 0.1 M neutral phosphate buffer. The abrasives were added directly to the inoculum and the tests were made on *Nicotiana glutinosa*.

The results presented in table 6 show that a great increase in number of lesions was brought about by all grades of the 2 abrasives. Carborundum of

TABLE 5.—*Influence of carborundum on the number of local lesions caused by tobacco-mosaic virus on the hybrid, Nicotiana tabacum × N. glutinosa, when applied by different methods. Inoculum diluted 1:10,000*

Experiment No.	Control	Method of applying carborundum and average number of lesions[a]		
		Added to inoculum	Sprinkled on leaves	Dusted on leaves
1	19.3	364.8	267.5	291.2
2	37.1	413.5	484.4	437.1
3	12.4	192.2	266.8	269.5
Average	22.9	323.5	339.6	332.6

[a] Average number of lesions per half-leaf on 30 half-leaves.

500-mesh effected the greatest increase in the 3 trials, being significantly different from the other grades. The other results with carborundum were not significantly different from one another. In the Aloxite series the lowest grade 280-mesh gave the highest number of lesions. The other results do not differ significantly. As a whole, the carborundum grades gave a greater increase than Aloxite. The increase in number of lesions for the 2 abrasives varied from 33 to 122 times the number of lesions obtained for the control.

The Use of Carborundum for Local-Lesion Tests with Cucumber-Mosaic Virus on Vigna sinensis var. Black

Plants of *Vigna sinensis* var. Black have been used for local-lesion tests with cucumber-mosaic virus (7). This test has not, however, been considered very dependable, the results of lesion counts being sometimes so low that they could not be used for estimating virus concentration. The use of carborundum when inoculating cucumber-mosaic virus to *Vigna* gave very good results. Not only was the number of lesions greatly increased, but the counts were uniform and always gave a relatively low standard deviation. Figure 2 gives an idea of the increase in number of lesions brought about by carborundum.

TABLE 6.—*Comparative effect of different grades of two abrasives on the number of local lesions caused by tobacco-mosaic virus on Nicotiana glutinosa. Inoculum 1/5 × 10-4 in 0.1 M phosphate buffer at pH 7*

Experiment No.	Treatment, grade of abrasive, and average number of lesions[a]										
	Control	280		320		400		500		600	
		Carbor.	Aloxite	Carbor.	Aloxite	Carbor.	Aloxite	Carbor.	Aloxite	Carbor.	Aloxite
1	0.3	22.8	.....	25.8	.....	39.6	.....	40.6	.....	37.1	.....
2	1.6	63.3	.....	76.0	.....	72.4	.....	92.6	.....	54.6	.....
3	0.5	73.2	.....	89.6	.....	100.2	.....	159.2	.....	112.4	.....
4	1.1	.....	32.8	.....	19.0	.....	31.4	.....	18.0	.....	20.0
5	0.5	.....	56.9	.....	36.5	.....	49.6	.....	39.5	.....	45.6
6	1.6	.....	90.7	.....	38.1	.....	55.5	.....	23.2	.....	43.2
Average	0.9	53.1	.....	63.8	.....	70.7	.....	97.5	.....	68.0	.....
	.....	.....	60.1	.....	31.2	.....	45.5	.....	26.9	.....	36.3

[a] Average number of lesions per half-leaf on 10 half-leaves.

TABLE. 7.—*Influence of carborundum on the number of local lesions caused by cucumber-mosaic virus on Vigna sinensis var. Black*

Diluent	Experiment No.	Dilution, treatment, and average number of lesions[a]							
		Undiluted		1: 5		1: 25		1: 125	
		Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
Distilled water	1	34.2	5.3	14.4	0.4	6.0	0.1	1.4	0.03
	2	75.0	6.6	37.1	0.7	20.6	0.1	4.7	0.0
	3	7.8	0.5	11.3	0.1	2.2	0.0	1.0	0.0
	Average	39.0	4.1	20.9	0.4	9.6	0.07	2.4	0.01
0.1 M neutral phosphate buffer	4	25.1	0.6	53.8	0.5	80.8	0.6	16.6	0.2
	5	64.8	0.9	233.7	5.1	19.5	0.2	1.9	0.0
	6	7.8	0.5	88.3	0.2	21.9	0.1	4.5	0.0
	Average	32.6	0.7	125.3	1.9	40.7	0.3	7.7	0.07

[a] Average number of lesions per leaf on 30 leaves, except for experiments 3 and 6, which are based on 20 leaves.

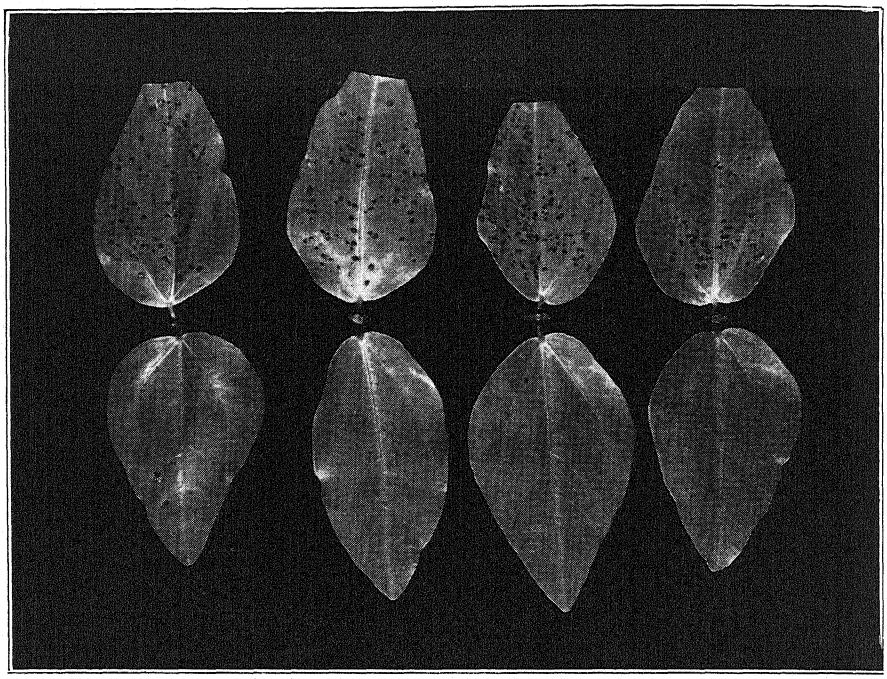


FIG. 2. Increase in the number of lesions obtained by use of carborundum. Plants representative of experiment 6, table 7, dilution 1: 5. (Photograph by J. A. Carlile.)

Two series of experiments were made with *Vigna*. Carborundum 320-mesh was used in both. In the first series distilled water was used as a diluent and in the second the dilutions were made with 0.1 M neutral phosphate buffer. The inoculum was taken from tobacco plants that had shown symptoms for 5 to 15 days.

Examination of the data presented in table 7 shows that the number of lesions obtained for the controls was very low, even when the inoculum was undiluted. In most experiments a satisfactory estimate of the virus concentration could not be based on the figures obtained for the controls. The use of carborundum increased greatly the number of lesions and permitted a comparison between the preparations of different concentrations. Table 8

TABLE 8.—*Standard deviation in percentage of the mean of local lesion tests made with cucumber-mosaic virus and carborundum on Vigna sinensis var. Black*

Experiment No.	Dilution, diluent, and standard deviation in percentage of the mean							
	Distilled water				0.1 M neutral phosphate buffer			
	Undiluted	1: 5	1: 25	1: 125	Undiluted	1: 5	1: 25	1: 125
1	16.1	11.8	15.5	27.9	11.3	7.6	6.4	8.9
2	11.5	11.8	15.5	24.0	10.5	5.6	9.7	15.8
3	16.7	16.8	22.7	30.0	16.7	7.9	10.5	13.3
Average	14.8	13.5	17.9	27.3	12.8	7.0	8.9	12.7

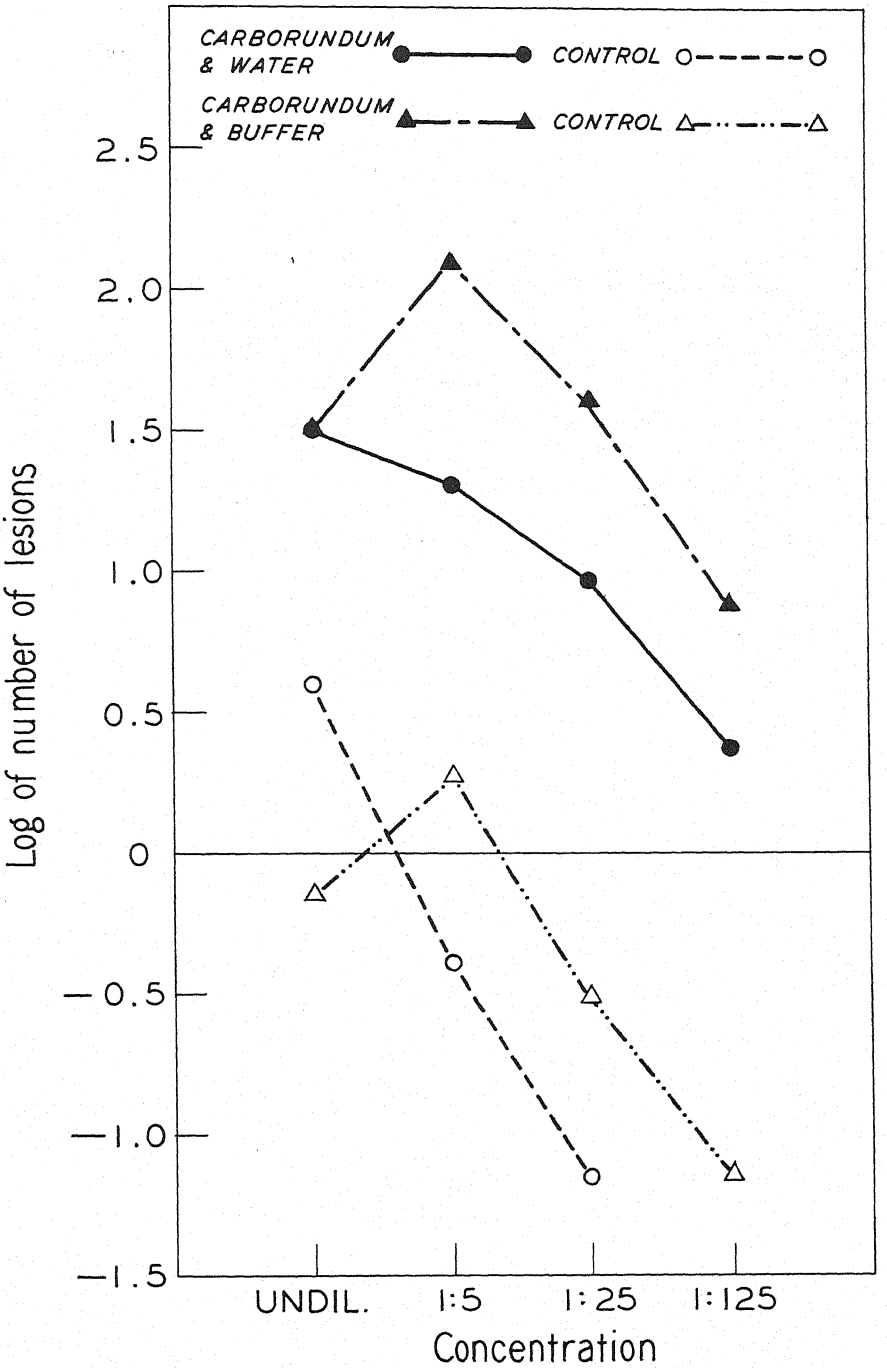


FIG. 3. Dilution curves of cucumber-mosaic virus on *Vigna sinensis* var. Black when inoculated with and without carborundum.

gives the standard deviation in percentage of the mean obtained in these experiments. The deviations are relatively small and permit fairly accurate comparisons.

It is interesting to note that the use of 0.1 M neutral phosphate buffer as a diluent increased the number of lesions in comparison with distilled water. It also decreased the standard deviation even if allowances are made for the greater number of lesions obtained. In the experiments performed with phosphate buffer as a diluent, the samples of virus diluted to 1:5 gave a significantly higher number of lesions than the undiluted juice. In one experiment a further dilution to 1:25 increased the number of lesions still more. This might suggest that the solutions were not properly buffered until the dilution had been increased to 1:25. In one of the experiments using distilled water, the preparation diluted to 1:5 also showed an increase over the undiluted inoculum. Figure 3 represents the curves obtained with and without the use of carborundum.

The increase in number of lesions due to the addition of 0.1 M neutral phosphate buffer is apparently to be attributed to the change of pH of the preparation. These results are, however, different from those obtained by Stanley (11). In his experiments, cucumber-mosaic virus was inactivated as the hydrogen-ion concentration was increased or decreased from pH 5.7.

It has been noticed that the use of carborundum in experiments with *Vigna* permits to a certain extent the use of plants of different ages without inconvenience.

Carborundum in Tests with Tobacco Severe-etch Virus on Physalis peruviana

Only a preliminary experiment was made with this virus. The results of the test made during the summer showed that the half-leaves inoculated without the abrasive had an average of 6.5 lesions per leaf, whereas the use of carborundum increased the average number of lesions to 123.8.

According to Holmes (5), it is difficult to obtain a satisfactory number of lesions in tests made during the summer. Under summer conditions the leaves of the plants are thicker and tougher than those grown in other seasons. Carborundum may prove of use in this situation, making the test feasible during the warm season.

DISCUSSION

It had already been shown by Samuel and Bald (10) that 120-mesh sand, used as an abrasive, increased the number of local lesions caused by a yellow-mottling strain of tobacco-mosaic virus on *Nicotiana glauca* Grah. and *N. tabacum* as tested by the iodine technique (4) and also on *N. glutinosa*. Rawlins and Tompkins (9) showed that the number of local lesions caused by tomato spotted-wilt virus on *N. glutinosa* was greatly increased by the use of carborundum. Black (1) reported good results with carborundum when inoculating potato yellow-dwarf virus on *N. rustica*. The experiments

herein reported with 3 viruses and 5 different host plants show that a great increase in the number of local lesions is obtained in these plants by the use of carborundum as an abrasive. Good results with carborundum in the transmission of certain diseases difficult to transmit mechanically have also been obtained by other authors in numerous cases. These results suggest that the entrance of all these viruses into the cells of various host plants when inoculated by rubbing involves the same or a similar mechanism. It seems very likely that carborundum or other abrasives also will increase the number of lesions for other viruses in other host plants.

Carborundum apparently renders the local-lesion tests more sensitive. This may be of little advantage with some viruses. However, under certain circumstances listed below its use may be profitable.

When submitting virus preparations to different treatments in which the titer is so decreased that accurate determinations cannot be made by ordinary means, the use of carborundum might permit such determinations.

Many viruses do not attain a high titer in plant juice. Unless they can be concentrated, their dilution curves cannot be studied satisfactorily by local-lesion tests. Carborundum increases the dilution range and would help in these cases.

The use of carborundum permits tests to be made with more dilute solutions. This at the same time dilutes other substances present in the inoculum and minimizes their possible interference. In testing mixtures of virus-inhibitor or virus-inactivator on dilution, carborundum may be useful.

In tests to determine end points, the use of carborundum might be advantageous.

It also seems advisable to use carborundum when searching for a host plant producing local lesions.

The writer wishes to thank Dr. L. O. Kunkel and Dr. F. O. Holmes for advice and criticism offered during this investigation and in the preparation of the present manuscript.

SUMMARY

Experiments with ordinary tobacco-mosaic virus on *Nicotiana tabacum* × *N. glutinosa*, *N. glutinosa*, and *Phaseolus vulgaris* var. Early Golden Cluster showed that the number of local lesions is greatly increased by the use of carborundum as an abrasive. The same is true for severe-etch virus on *Physalis peruviana* and for cucumber-mosaic virus on *Vigna sinensis* var. Black.

The local-lesion test with cucumber-mosaic virus on *Vigna sinensis* var. Black is greatly improved by use of carborundum, which permits the estimation of virus concentration in samples that could not be measured otherwise. The use of 0.1 M neutral phosphate buffer as a diluent for cucumber-mosaic virus also increased the number of lesions in comparison with distilled water and decreased the standard deviation. Preparations diluted to 1:5

with 0.1 M phosphate buffer gave a higher number of lesions than the undiluted preparations.

Carborundum has no action on tobacco-mosaic virus. Samples to which carborundum was added and removed behaved like the controls.

Three methods of applying carborundum (dusting, sprinkling, and adding to the juice) gave apparently the same results with tobacco-mosaic virus on the hybrid, *Nicotiana tabacum* × *N. glutinosa*.

Five grades of 2 abrasives varying from 280-mesh to 600-mesh were tried. Carborundum 500-mesh gave the best results among the different grades of this abrasive, as did Aloxite 280-mesh in the Aloxite series. However, the results are more or less approximate and any of the grades of either carborundum or Aloxite effected a large increase in number of lesions as compared with the controls on *Nicotiana glutinosa*. As a whole, the carborundum grades gave a higher number of lesions than Aloxite.

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STUDIES OF STEM RUST (PUCCINIA GRAMINIS) FROM POA AMPLA, AVENA FATUA, AND AGROPYRON SPICATUM IN THE PULLMAN, WASHINGTON, REGION[1]

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INTRODUCTION

In the Pacific Northwest during recent seasons stem rust (*Puccinia graminis* Pers.) has been a destructive disease of grasses and cereals. During the past several years reports have been made on the incidence of disease in native and introduced grasses in the Pacific Northwest, and stem rust has always been prominent among the diseases reported (10, 11, 23). In the most extensive of these reports on grass diseases in certain western States (13), 81 species and varieties of grasses were listed as showing more or less infection by stem rust in 1941 alone. It seems probable that more than 100 species of grasses and cereals serve as hosts to *Puccinia graminis* in the Pacific Northwest. It is doubtful if any other region of the United States has stem rust on as wide a range of host species. In spite of this, however, surprisingly little work has been done on host relationships, variety identification, and physiologic race surveys in the stem rust problem in the Pacific Northwest.

In 1940, late-summer and early-fall weather conditions contributed to epiphytotic development of stem rust on many grasses in the Pullman, Washington, area, especially *Agropyron, Elymus,* and *Poa* spp. At this time collections were made of stem rust on wild oats (*Avena fatua* L.), blue bunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and Smith), and big bluegrass (*Poa ampla* Merr.) for greenhouse study during the fall, winter, and spring (1940–41). Of especial interest was the stem rust on bluegrasses, which was reported on twelve *Poa* spp. (11). Only one variety of stem rust (*P. graminis poae*) has been known to be commonly associated with *Poa* spp. and this only in Europe, Russia, and a limited region in the central States of this country (28). In 1937 D. C. Smith[3] sent, among other stem-rust collections made at Pullman, a collection on *P. nevadensis* to M. N. Levine,

[1] Investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, in cooperation with the Soil Conservation Service, Division of Nurseries, U. S. Department of Agriculture, and the Washington State Agricultural Experiment Station, Pullman, Washington. Published with the approval of the Director as Scientific Paper No. 555.

[2] Associate Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and Assistant Agronomist, Nebraska Agricultural Experiment Station, respectively. Junior writer formerly Graduate Assistant, Division of Agronomy, Washington State Agricultural Experiment Station. The writers are indebted to M. N. Levine, Pathologist, Division of Cereal Crops and Diseases, for valuable suggestions regarding the interpretations of the data and the preparation of the manuscript.

[3] Formerly of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. D. A.

Pathologist, U. S. Dept. of Agriculture, stationed at University Farm, St. Paul, Minn. Dr. Levine identified the collection as *P. graminis avenae* race 2. This is only the second record of a *Poa* spp. harboring any variety but *P. graminis poae*.[4] It was obvious, then, that the stem rust prevalent on *Poa* spp. and other hosts in the Pullman area merited further study.

MATERIALS AND METHODS

Single-spore cultures of 3 stem-rust collections were obtained by increasing inoculum from a well-isolated sorus of each of these rusts on a sparsely infected, highly susceptible host. The rust from *Poa ampla* was propagated on plants of this species; the rust from *Avena fatua* was propagated on seedlings of Markton oats (C.I. 2053); and that from *Agropyron spicatum* on *Elymus glaucus* Buckl.

The various species and accessions of grasses[5] tested were grown in the greenhouse in 2½-in. pots. Each pot contained 5–15 plants. Inoculations with the 3 cultures were performed in different corners of the same greenhouse, and to some extent in different greenhouses. Whenever the reading for any given accession was similar for more than one of the rust collections, indicating possible contaminations, several accessions of grasses known to be susceptible to only one collection were inoculated with the rust in question. Inoculations were made by spraying fresh spore suspensions with an atomizer or by direct application of spores from heavily infected highly susceptible plants. The inoculated plants were incubated 30–48 hours under several layers of wet sheeting. Infection data were taken 14–16 days after inoculation and classified according to type of infection from 0–4, as described by Stakman and Levine (27).

Most of the grasses employed in these studies are listed in the tables in the following pages. Since it is not practicable to give authorities for binomials listed in the tables and, since immune species are not included therein, a complete list of the species tested follows:[6]

Agropyron caninum (L.) Beauv., *A. ciliare* (Trin.) Franch, *A. cristatum* (L.) Gaertn., *A. dasystachyum* (Hook.) Scrib., *A. elmeri* Scribn., *A. desertorum* (Fisch.) Schult., *A. elongatum* (Host) Beauv., *A. inerme* (Scribn. and Smith) Rydb., *A. intermedium* (Host) Beauv., *A. repens* (L.) Beauv., *A. semicostatum* (Steud.) Nees, *A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb., *A. spicatum* (Pursh) Scribn. and Smith, *A. subsecundum* (Link) Hitchc., *A. trachycaulum* (Link) Malte, *A. trichophorum* (Link) Richt.; *Agrostis alba* L., *A. castellana* Boiss. and Reut., *A. exarata* Trin., *A. scabra* Willd., *A. palustris* Huds., *A. scabriglumis* Boiss. and Reut., *A. spicaventi* L., *A. stolonifera* L., *A. tenuis* Sibth.; *Alopecurus pratensis* L.; *Arrhenatherum elatius* (L.) Presl.; *Bromus arvensis* L., *B. brevis* Nees, *B. brizaeformis* Fisch. and May., *B. carinatus* Hook. and Arn., *B. catharticus*

[4] Stakman (24) successfully transferred stem rust from *Poa nemoralis* to oats, while Stakman *et al.* (29) isolated physiologic races 1 and 2 from *Poa trivialis* collected at Lafayette, Indiana, in 1921.

[5] Mostly contributed by the Pullman nursery unit of the Soil Conservation Service.

[6] Insofar as possible the nomenclature given by Hitchcock (14) is followed.

Vahl., *B. commutatus* Schrad., *B. erectus* Huds., *B. inermis* Leyss., *B. japonicus* Thunb., *B. macrostachys* Desf., *B. macrostachys* var. *lanuginosus* (Poir.) Cos. and Dur., *B. madritensis* L., *B. mollis* L., *B. purgans* L., *B. rigidus* Roth., *B. rubens* L., *B. secalinus* L., *B. squarrosus* L., *B. tectorum* L., *B. tomentellus* Boiss., *B. vulgaris* (Hook.) Schear; *Dactylis glomerata* L.; *Danthonia pilosa* R. Br., *D. semiannulosus* (Labill.) R. Br., *D. unispicata* (Thurb.) Munro; *Deschampsia caespitosa* (L.) Beauv., *D. elongata* (Hook.) Munro; *Elymus canadensis* L., *E. glaucus* Buckl., *E. triticoides* Buckl., *E. villosus* Muhl., *E. virginicus* L., *E. virginicus* var. *intermedius* (Vasey) Bush; *Festuca elatior* L., *F. idahoensis* Elmer, *F. obtusa* Spreng., *F. occidentalis* Hook., *F. octoflora* Walt., *F. ovina* L., *F. rubra* L., *F. scabrella* Torr., *F. Thurberi* Vasey, *F. viridula* Vasey; *Glyceria elata* (Nash) Hitchc., *G. grandis* S. Wats., *G. pauciflora* Presl., *G. striata* (Lam.) Hitchc.; *Holcus lanatus* L.; *Hordeum brevisubulatum* (Trin.) Link, *H. bulbosum* L., *H. gussonianum* Parl., *H. jubatum* L., *H. jubatum* var. *caespitosum* (Scribn.) Hitch., *H. nodosum* L.; *Koeleria cristata* (L.) Pers.; *Lolium perenne* L.; *Phalaris arundinacea* L.; *Phleum phleoides* (L.) Karst., *P. pratense* L.; *Poa ampla* Merr., *P. annua* L., *P. arida* Vasey, *P. attenuata* Trin., *P. bulbosa* L., *P. canbyi* (Scribn.) Piper, *P. compressa* L., *P. curta* Rydb., *P. cusickii* Vasey, *P. epilis* Scribn., *P. glaucifolia* Scribn. and Will., *P. gracillima* Vasey, *P. interior* Rydb., *P. juncifolia* Scribn., *P. nemoralis* L., *P. nervosa* (Hook.) Vasey, *P. nevadensis* Vasey, *P. palustris* L., *P. pratensis* L., *P. scabrella* (Thurb.) Benth., *P. secunda* Presl., *P. sphondylodes* Trin., *P. stenantha* Trin., *P. sterilis* Bieb., *P. sylvestris* A. Gray; *Sitanion hystrix* (Nutt.) J. G. Smith, *S. jubatum* J. G. Smith; *Trisetum flavescens* (L.) Beauv., *T. spicatum* (L.) Richt.

The following cereals were included: *Avena byzantina* C. Koch, *A. fatua* L., *A. nuda* L., *A. sativa* L.; *Secale cereale* L., *Triticum aestivum* L.

The biometrical studies of the urediospores of the 3 stem-rust collections used in the present experiments are based on lengths and widths of 150 spores of each collection. Care was taken to obtain only the superficial spore layers of several mature rust pustules. The spores were mounted in hot lacto-phenol.

RESULTS

One hundred and twenty-three species of grasses and cereals, in 23 genera, were used in the host-range studies of the 3 collections of *Puccinia graminis* from *Agropyron spicatum, Avena fatua,* and *Poa ampla.* A tabular summary of the general reaction of these 23 genera to the 3 stem-rust collections is given in table 1.

A glance at table 1 shows that the strain of *Puccinia graminis* from *Poa ampla* has a surprisingly extensive host range. Within 14 genera, 34 species of grasses and cereals showed more or less susceptibility. Less extensive is the host range of the strain of stem rust from wild oats. Here 18 species in 9 genera proved susceptible. In marked contrast is the very narrow host

range of the stem rust from *Agropyron spicatum* to which 7 species in only 3 genera are susceptible, and these are all of the same tribe (Hordeae).

An analysis of the comparative reaction of the numerous species and

TABLE 1.—*General reactions of 23 genera of grasses and cereals to stem rust collected locally on Poa ampla, Avena fatua, and Agropyron spicatum, near Pullman, Washington*

Reaction of genera	Source of collections					
	Poa ampla		*Avena fatua*		*Agropyron spicatum*	
		No. spp.		No. spp.		No. spp.
Resistant (Infection type 0–2)	*Agropyron*	17	*Agropyron*	17	*Agropyron*	13
	Agrostis	6	*Agrostis*	9	*Agrostis*	9
	Arrhenatherum	1	*Alopecurus*	1	*Alopecurus*	1
	Avena	2	*Bromus*	20	*Arrhenatherum*	1
	Bromus	20	*Danthonia*	3	*Avena*	1
	Danthonia	3	*Elymus*	6	*Bromus*	21
	Elymus	6	*Festuca*	7	*Dactylis*	1
	Festuca	6	*Glyceria*	2	*Danthonia*	3
	Holcus	1	*Holcus*	1	*Deschampsia*	2
	Hordeum	5	*Hordeum*	6	*Elymus*	5
	Lolium	1	*Lolium*	1	*Festuca*	10
	Phleum	1	*Phleum*	2	*Glyceria*	2
	Poa	15	*Poa*	26	*Holcus*	1
	Secale	1	*Secale*	1	*Hordeum*	6
	Sitanion	2	*Sitanion*	2	*Koeleria*	1
	Trisetum	1	*Triticum*	1	*Lolium*	1
	Triticum	1			*Phalaris*	1
					Phleum	2
					Poa	25
					Secale	1
					Trisetum	2
					Triticum	1
Susceptible (Infection type 3–4)	*Agrostis*	3	*Arrhenatherum*	1	*Agropyron*	4
	Alopecurus	1	*Avena*	4	*Elymus*	1
	Avena	2	*Dactylis*	1	*Sitanion*	2
	Bromus	1	*Deschampsia*	2		
	Dactylis	1	*Festuca*	3		
	Deschampsia	2	*Koeleria*	1		
	Festuca	3	*Phalaris*	1		
	Glyceria	3	*Poa*	3		
	Hordeum	1	*Trisetum*	2		
	Koeleria	1				
	Phalaris	1				
	Phleum	1				
	Poa	13				
	Trisetum	1				
Totals (susceptible)		34		18		7

accessions of grasses and cereals included in this experiment to the three stem-rust collections is given in table 2.

The data in table 2 emphasize those in table 1, showing further the considerable host range differences between the 3 collections of stem rust used in these studies. The range of the Poa rust is especially surprising, since it included grasses in 5 different tribes: *Phalaridae, Avenae, Festucae, Agrostideae,* and *Hordeae.* In contrast, only 1 tribe (*Hordeae*) is repre-

TABLE 2.—*Comparative reaction of various grasses and cereals to cultures of Puccinia graminis isolated from Poa ampla, Avena fatua, and Agropyron spicatum, near Pullman, Washington*

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
Agropyron				
dasystachyum	P 7801[a]	0	0	3
elmeri	P 1824	0	2	2
inerme	P 2697	0	1+	0
inerme	P 2792	0	0	4
inerme	P 2522	0	0	2
smithii	F 279	0	0	3
spicatum	P 3548	0	0	4
spicatum	P 2719	0	0	4
trachycaulum	F 6	0	0	1
trachycaulum	P 5365	0	0	1
Agrostis				
alba	P 2431	4	0	0
alba	P 2429	4	0	0
alba	P 78	3	0	0
castellana	P 2328	2	0	0
exarata	P 750	3	0	0
palustris	P 4462	2+	2	0
spica-venti	P 75	3	0	0
stolonifera	P 77	2	0	0
striata	P 3176	2+	0	0
tenuis	P 2434	2	0	0
Alopecurus				
pratensis	P 124	4	2	0
Arrhenatherum				
elatius	P 3052	2	4	0
elatius	P 4295	2	4	0
elatius	F 78	0	3	0
elatius	P 1719	0	4	0
Avena				
byzantina				
"Nortex"	C.I. 2382	4	4	–
fatua		4	4	–
nuda				
"Chinese Hulless"		0	4	–
sativa				
"Anthony"	C.I. 2143	0	4	–
Bannock	C.I. 2592	0	4	–
Boone	C.I. 3305	0	1+	–
Fulton	C.I. 3327	0	4+	–
Green Russian	C.I. 2344	0	4	–
Hancock	C.I. 3346	0	1	–
Joanetta	Wn. 2331	0	4	–
Marion	C.I. 3247	0	1	–
Markton	C.I. 2053	0	4	0
Marida	C.I. 2571	0	4	–
Rainbow	C.I. 2345	0	1	–
Red Rustproof	C.I. 458	0	4	–
Iowar	C.I. 847	0	4	–
Victory	C.I. 1197	0	4	–

[a] "P" accessions are those of the Pullman Nursery Unit of the Soil Conservation Service.

"Wn." accessions are those of the Washington Agricultural Experiment Station.

"F" accessions are those of the senior author.

C.I. accessions are those of the Division of Cereal Crops and Diseases, Bureau of Plant Industry.

TABLE 2—(*Continued*)

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
Bromus				
brizaeformis	P 2578	2+	1	0
carinatus	P 2725	1	0	0
commutatus	F 193	2+	2	0
macrostachys	F 151	2	0	0
macrostachys var. *lanuginosus*	F 152	2+	2	0
madritensis	F 150	1+	0	0
mollis	F 153	2	2	0
purgans	F 354	1	–	–
rigidus	F 275	1+	1+	0
rubens	F 146	3	1	0
squarrosus	F 143	2	1	0
tectorum	F 165	1+	1+	0
Dactylis				
glomerata	P 187	4	4	0
glomerata	P 952	4	3	0
Deschampsia				
caespitosa	P 779	4	4	0
elongata	P 956	4	4	0
Elymus				
glaucus	F 127	0	0	4
glaucus	P 1848	0	0	4
glaucus	P 1851	0	0	4
glaucus	P 2662	0	–	4
glaucus	P 3572	0	0	4
glaucus	P 3562	1	0	–
sibiricus	P 214	0	0	1
triticoides	P 2714	2	0	0
virginicus	F 133	0	0	1
Festuca				
idahoensis	P 2807	3	0	0
octoflora	P 3130	4	4	0
rubra	P 2347	3	0	0
thurberi	P 5521	2+	3	0
viridula	P 5060	–	3	0
Glyceria				
elata	P 1816	3	–	–
grandis	P 4970	4	0	0
striata	P 3719	3	–	–
Hordeum				
brevisubulatum	P 303	1+	0	0
gussonianum	F 116	2+	1+	2
gussonianum	F 118	2+	0	1
jubatum var. *caespitosum*	F 334	1+	0	1
nodosum	F 122	1+	0	0
nodosum	F 169	2+	0	0
nodosum	P 2723	3	0	0
Koeleria				
cristata	P 1875	4	3	0
cristata	P 2648	4	3+	0
Phalaris				
arundinacea	P 2368	4	3+	0
Phleum				
phleoides	P 2490	3	1	0

TABLE 2—(*Continued*)

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
Poa				
ampla	38 PR 395[b]	4	2	0
ampla	38 PR 407	4	1	0
ampla	38 PR 411	4	0	0
ampla	38 PR 419	4	2	0
ampla	38 PR 493	4	0	0
ampla	38 PR 749	4	3	0
ampla	None	4	1	0
ampla	P 846	4	2	0
ampla	P 910	4	1+	0
ampla	P 914	4	1	0
ampla	P 996A	4	2+	0
ampla	P 996B	4	2	0
ampla	P 999	4	2+	0
ampla	P 1000	4	1	0
ampla	P 1880	4	2	0
ampla	P 1883	4	0	0
ampla	P 1888	4	0	0
ampla	P 2534	3	0	0
ampla	P 2716	4	0	0
ampla	P 2800	4	0	0
ampla	P 3317–4	4	0	0
ampla	P 3446	4	0	0
ampla	P 3482	4	0	0
ampla	P 3935	4	0	0
ampla	P 5002	4	0	0
ampla	P 5113	4	1	0
ampla	P 5731	4	0	0
ampla	P 5732	4	0	0
ampla	P 5895	4	0	0
ampla	P 6239	4	2	0
ampla	P 6252	4	0	0
ampla	P 6303	4	1	0
ampla	P 8903	4	2	0
arida	P 5110	3+	0	0
attenuata	P 405	4	0	0
bulbosa	P 2788	4	4	0
bulbosa	P 5011	2	3+	0
bulbosa	P 5727	4	4	0
canbyi	38 PR 399	3	1+	0
canbyi	38 PR 453	3	0	0
canbyi	38 PR 759	4	1	0
canbyi	38 PR 760	2+	0	0
canbyi	38S 1535–1536	3	0	0
canbyi	38S 1539–1540	2+	0	0
canbyi	38S 1541–1542	2+	0	0
canbyi	38S 1543–1544	3	1	0
canbyi	38S 1547–1548	2+	0	–
canbyi	P 844	3+	0	0
canbyi	P 1884	4	2	0
canbyi	P 2756	4	0	0
canbyi	P 3321	4	2+	0
canbyi	P 4052	4	1	0
canbyi	P 6235	4	2	0
canbyi	P 7646	4	0	0
curta	P 2742	4	1	0
cusickii	P 906	2	0	0
cusickii	P 1886	2	0	0

[b] Accessions with designations of this sort are selections made by D. C. Smith, formerly of the Bureau of Plant Industry.

TABLE 2—(*Continued*)

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
epilis	P 2743	3	0	0
glaucifolia	P 5506	4	4	0
gracillima	P 850	4	1	0
gracillima	P 1904	3+	1	0
interior	P 5502	1	0	0
juncifolia	38 PR 401	4	2+	0
juncifolia	38 PR 403	4	1	0
juncifolia	38 PR 404	4	0	0
juncifolia	38 PR 405	4	2	0
juncifolia	38 PR 406	4	0	0
juncifolia	38 PR 527	4	1	0
juncifolia	P 837	4	0	0
juncifolia	P 1882	4	2	0
juncifolia	P 1884	4	0	0
nervosa	P 1894	1	1	0
nervosa	P 1898	2	–	0
nevadensis	38 PR 486	4	2+	0
nevadensis	P 5014	4	–	0
nevadensis	P 5017	3+	0	0
nevadensis	P 6281	3	2	0
pratensis	P 426	1	0	0
scabrella	P 2680	3	0	0
scabrella	P 2682	3	2	0
secunda	P 3795	3	0	0
secunda	P 4858	3	2	0
secunda	P 5711	3	1+	0
sphondylodes	P 3021	1	0	0
Secale				
cereale	bulk R. no No.	0	1+	1
"Selection 2B"		0	0	0
"Omskaia"		0	0	0
"Yelislgen"		0	0	0
Sitanion				
hystrix	F 278	0	0	3
jubatum	P 3360	0	0	4
Trisetum				
flavescens	P 461	1	3+	0
spicatum	P 2381	4	4	0
Triticum				
aestivum				
11 varieties[c]		0	0	0
Other species[d]		0	0	0

[c] Athena (C.I. 11693), Federation (C.I. 4734), Hybrid 128 (C.I. 4512), Jenkin (C.I. 5117), Kharkof (C.I. 1442), Oro (C.I. 8220), Requa (C.I. 1554), Rex (C.I. 10065), Tenmarq (C.I. 6939), Turkey (C.I. 6175), Yogo (C.I. 8033).

[d] The species that proved to be immune from all 3 of the stem-rust cultures are as follows: *Agropyron caninum, A. ciliare, A. cristatum, A. desertorum, A. elongatum, A. intermedium, A. repens, A. semicostatum, A. sibiricum, A. subsecundum, A. trichophorum; Agrostis scabra, A. scabriglumis; Bromus arvensis, B. brevis, B. catharticus, B. erectus, B. inermis, B. japonicus, B. secalinus, B. tomentellus, B. vulgaris; Elymus canadensis, E. triticoides, E. villosus, E. virginicus* var. *intermedius; Festuca elatior, F. obtusa, F. occidentalis, F. ovina, F. rubra, F. scabrella; Glyceria pauciflora; Holcus lanatus; Hordeum bulbosum, H. jubatum; Lolium perenne; Phleum pratense; Poa annua, P. compressa, P. nemoralis, P. palustris, P. pratensis, P. stenantha, P. sterilis, P. sylvestris.*

sented in the very limited host range of the stem-rust culture from *Agropyron spicatum.* Some evidence of parallelism may be noticed between the rust from *Poa ampla* and that from *Avena fatua.* However, the two are, nevertheless, sharply distinguished by the reaction of such species as *Agrostis alba, Poa ampla, P. canbyi, P. juncifolia,* and wild and cultivated oats. The Poa rust is innocuous on oats and the oat rust is similarly weak on most of the *Poa* spp.

It is worthy of note that, although nearly 40 accessions of *Poa ampla* were included in these experiments, none showed any resistance. This was disappointing, since the heavy infestations of stem rust in plots and increase fields of this grass had prompted an attempt to find resistant accessions that might be substituted for the highly susceptible accessions now being used. On the basis of the results obtained it seems unlikely that any marked resistance of *P. ampla* to stem rust will be obtained by selection.

Comparative Morphology

Since it has been shown (17) that spore morphology can be used as additional evidence in the identification of varieties of *Puccinia graminis,*

TABLE 3.—*A comparison of lengths and widths of urediospores of the varietal complexes of Puccinia graminis with those of three collections of stem rust from Agropyron spicatum, Avena fatua, and Poa ampla*

Varietal complexes or isolates from local stem rust collections	Mean		Number of measurements
	Length	Width	
Rust varieties reported in literature:			
P. g. tritici (17)[a]	32.40 ± 0.19	19.79 ± 0.06	200
secalis (17)	27.14 ± 0.14	17.19 ± 0.06	200
avenae (17)	28.50 ± 0.15	19.94 ± 0.07	200
phleipratensis (17)	23.95 ± 0.12	16.88 ± 0.06	200
agrostidis (17)	22.37 ± 0.12	15.68 ± 0.05	200
poae (28)	18.64 ± 0.10	15.78 ± 0.07	100
Rust strains isolated from:			
Agropyron spicatum	29.35 ± 0.13	16.98 ± 0.09	150
Avena fatua	25.98 ± 0.09	17.08 ± 0.06	150
Poa ampla	24.81 ± 0.11	16.18 ± 0.06	150

[a] Refers to Literature cited.

biometrical studies were made of the urediospores of the 3 cultures of stem rust. One hundred and fifty mature spores of each collection on a highly susceptible host were measured for length and width. A comparison of the measurements thus obtained with the established measurements (17, 27) of the 6 varieties of *Puccinia graminis* recognized as occurring in the United States, is shown in table 3. As will be mentioned later the collections of stem rust on which the present paper is based do not conform biometrically to any of the 6 varieties of *P. graminis.* However, since Levine (18) and Waterhouse (37) have shown that some physiologic races within the rust variety *tritici* differ significantly in spore size and Bailey (1) and Waterhouse (37) have demonstrated the same thing in the case of *P. graminis*

avenae, these local collections may quite possibly be within the range of variation expected for the various physiologic races of the several varieties of *P. graminis.*

DISCUSSION

In view of the host ranges of the 3 collections of stem rust included in these studies the question of varietal identity becomes of great interest.

The stem rust culture from *Poa ampla* obviously is not *Puccinia graminis poae,* as commonly known. For this variety it has been shown by Eriksson, (4, 5, 6, 7), Eriksson and Henning (9), Jaczewski (15), and Stakman and Levine (28) that only a limited number of *Poa* spp.[7] serve as congenial hosts. Attempts to infect grasses in several other genera have failed. The present work with the single spore culture of stem rust from *P. ampla* shows conclusively that a large number of *Poa* spp. are among its hosts. Several of the species shown by previous investigators to be hosts to *P. graminis poae* were included but all were either immune from or highly resistant to the *P. ampla* rust. It also has been shown in the present investigations that many other species, in 13 other genera, are also more or less susceptible to this polyvorous strain of *P. graminis.* Many of these species and genera have been shown by several investigators[8] to be susceptible to *P. graminis avenae.* The complete susceptibility of Nortex oats (*Avena byzantina*) further suggests the variety *avenae.* However, the demonstrated immunity of 16 other varieties of cultivated oats, including Markton and Victory, strongly indicates a new physiologic race of *P. graminis avenae.*[9] The susceptibility of certain species of *Agrostis, Alopecurus, Dactylis,* and *Koeleria,* listed by Fischer and Levine (12) as hosts to *P. graminis agrostidis* suggests the possibility that the *P. ampla* rust might belong to that variety. On the other hand, the *P. ampla* rust has certain hosts not recognized for *P. graminis agrostidis,* namely, *Avena byzantina, A. fatua, Deschampsia* spp., *Festuca* spp., *Glyceria* spp., *Hordeum nodosum, Phalaris arundinacea, Phleum phleoides, Poa* spp., and *Trisetum spicatum.* The host range also has certain species in common with *P. graminis phlei-pratensis* (31, 33), but the immunity of the 3 accessions of *Phleum pratense* from the stem rust from *Poa ampla* would seem to preclude the possibility of this rust being *P. graminis phlei-pratensis.* By no stretch of the imagination could the *P. ampla* rust be considered as *P. graminis tritici* or *P. graminis secalis.*

A comparison of the urediospore morphology of the *Poa ampla* rust with the established biometric constants (17) of the 6 varieties of *Puccinia graminis* in the United States (Table 3) does not aid in determining the variety of stem rust concerned. The measurements of the rust in question do not conform to those of any of the varieties of *P. graminis.* The nearest

[7] *Poa annua, P. chaixii* Vill., *P. caesia* Smith, *P. compressa, P. fertilis, P. nemoralis, P. palustris, P. pratensis, P. serotina* Ehrh., *P. triflora* Gilib. (The latter two are cited by Hitchcock (14) as synonyms of *P. palustris.*)

[8] See footnote 11.

[9] According to correspondence with M. N. Levine this new race would have the key number 14.

approach is to *P. graminis phlei-pratensis* and *P. graminis agrostidis* but, as already pointed out, these varieties are excluded because of marked host range differences.

It seems, then, that the culture of stem rust from *Poa ampla* might be identified with the variety *Puccinia graminis avenae*. Other varieties of *P. graminis* have been named in Europe and Russia[10] but the validity of at least some of these seems to be in question, and insufficient is known of their host range to permit comparison with the *Poa* rust in question.

A comparison of the present results on stem rust from *Avena fatua* with the work of previous investigators leaves no doubt that it belongs to *Puccinia graminis avenae*. All but 3 of the 17 varieties and species of oats were susceptible. The susceptible reaction of species of *Arrhenatherum, Dactylis, Deschampsia, Festuca, Koeleria, Phalaris, Poa,* and *Trisetum* are largely in accord with the earlier published records for *P. graminis avenae*.[11] What differences do exist can easily be attributed to variations in grass host range of the various physiologic races within *P. graminis avenae*.

TABLE 4.—*The reactions of Puccinia graminis avenae host differentials when inoculated with stem rust collected on Avena fatua at Pullman, Washington*

Variety	C.I. No.	Infection type
White Tartar	551	2
Richland	787	1+
Sevenothree	3251	4

The identity of the physiologic race of *Puccinia graminis avenae* isolated from *Avena fatua* seemed worthy of determination. The 3 stem-rust differential oat varieties used by Levine and Smith (19) were obtained and inoculated with the single spore culture of stem rust from *A. fatua*. The reaction of these varieties is shown in table 4.

The resistant to moderately resistant reaction of Richland and White Tartar and the high susceptibility of Sevenothree point to physiologic race 2, in the light of the analysis and summary of the 10 known races of oats stem rust given by Levine and Smith (19). These authors show a mean reaction of 2++ for White Tartar to race 2, and in the present studies the reaction of White Tartar to the stem rust was 2. However, this does not seem to be significantly at variance with previous work (19), and, with Richland resistant and Sevenothree completely susceptible, there apparently is no other known race to which the collection of stem rust in question could belong.

The very narrow infection range of the culture of stem rust from *Agropyron spicatum* makes it impossible definitely to identify it with any known variety of *Puccinia graminis*. Only 4 species of *Agropyron,* 1 of *Elymus,* and 2 of *Sitanion* proved susceptible. When the original field

[10] *P. g. airae* Erikss. and Henn. (8), *P. g. calamagrostidis* Jacz. (15), *P. g. aperae* Jacz. (15), *P. g. arrhenatheri* Jacz. (15).

[11] See Literature cited: 1, 2, 5, 6, 7, 8, 12, 15, 16, 22, 25, 30, 31, 32, 33, 34, 35.

collection on *Agropyron spicatum* was brought into the greenhouse the inoculum was sown on *Elymus glaucus* and Oro and Federation wheat. A mesothetic reaction resulted on the wheat varieties, and a completely susceptible (infection type 4) reaction on the *Elymus* species. On the basis of these results it was then supposed that some race of *P. graminis tritici* was involved. Plants of the same accession of *Elymus glaucus* were later lightly infected with the stem rust increased on *E. glaucus* and one of the resulting pustules was chosen as the basis for the single-spore culture. However, as already noted, this single-spore culture was unable to infect any of the 12 wheat varieties, and these were twice inoculated in the seedling stage. Furthermore, Oro and Federation, which had reacted mesothetically to the original composite collection from *Agropyron spicatum,* were repeatedly inoculated in the seedling stage with the single-spore culture, but each time no noticeable infection resulted. From these results it seems probable that *P. graminis tritici* was represented in the original composite collection from *Agropyron spicatum* but not in the single-spore culture propagated on *Elymus glaucus,* the identity of which is, therefore, still in question. On the basis of earlier records (12) the only other known variety to which the single-spore culture could belong is *P. graminis secalis.* However, the resistance or immunity of *Agropyron repens* and 3 rye varieties, as well as one lot of bulk unpedigreed rye, while not entirely precluding the possibility of *P. graminis secalis,* certainly is not in support of that variety.

Numerous investigators[12] have cited many species of *Agropyron, Bromus, Elymus, Hordeum,* and to a lesser extent other genera as susceptible to *P. graminis tritici* and *P. graminis secalis.* The very limited grass-host range of the single-spore culture of stem rust from *Agropyron spicatum* thus makes it seem even more improbable that either of these two stem-rust varieties is concerned. Rather, it seems more likely that a new variety of *P. graminis* may have been encountered that is highly specialized to *Elymus glaucus* and a few species of *Agropyron* and *Sitanion.*

The results described in this paper indicate the presence of anomalous strains of stem rust in the Pacific Northwest. The moderate abundance of barberry bushes in this area provides adequate opportunity for new strains to have developed by hybridization. It seems worthwhile and desirable that intensive study be made of stem rust occurring in this region on a very wide range of grasses, and such investigations are already in progress.

SUMMARY

The results are given of inoculations of numerous grasses and cereals with single-spore cultures of stem rust from *Avena fatua, Poa ampla,* and *Agropyron spicatum* collected in the vicinity of Pullman, Washington.

The 3 cultures of stem rust varied widely in their host ranges: to the *Poa* rust culture, 34 species of grasses and cereals showed more or less susceptibility and these represented 14 genera and 5 tribes; to the *Avena fatua*

[12] See Literature cited: 2, 3, 4, 5, 6, 7, 8, 12, 15, 20, 21, 22, 25, 26, 30, 31, 32, 33, 34, 35, 36.

culture, 18 species in 9 genera and 3 tribes were more or less susceptible; to the *Agropyron* culture only 7 species were susceptible, representing 3 genera and only 1 tribe.

The culture of stem rust from *Avena fatua* was identified as physiologic race 2 of the variety *Puccinia graminis avenae.*

The culture from *Poa ampla* seems best identified with the variety *Puccinia graminis avenae,* but the demonstrated immunity of 16 out of 17 varieties of cultivated oats, including Markton and Victory, would seem to indicate a new physiologic race. It represents a virulent and polyvorous strain, but primarily on grasses.

The culture from *Agropyron spicatum* does not seem to belong to any known variety of *Puccinia graminis.* The immunity of 3 accessions of *Agropyron repens* and 3 of rye indicates that *P. graminis secalis* was not represented, and the immunity of 12 wheat varieties indicates that the variety *tritici* was not involved.

Biometrically, the urediospores of the 3 collections did not conform to the established biometric constants of any of the varietal complexes of *Puccinia graminis,* but might, nevertheless, be component part of some of them.

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CHEMICAL AND PHYSICAL CHARACTERISTICS OF MAIZE COBS IN RELATION TO THE GROWTH OF NIGROSPORA ORYZAE[1]

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Poorly matured ears of maize were found more frequently attacked by *Nigrospora oryzae* (B. and Br.) Petch than were well-matured ears. Nigrospora infection in maize ears usually was initiated in the cob, the fungus later invading the kernels. Except for the relationship between cob pH and incidence of disease discovered by Reddy (4) and the observation by Savulescu and Rayss (5) that susceptible ears contained more moisture in the cob under field conditions than resistant ears, apparently no work has been reported on the nature of cobs in relation to incidence of disease.

Arzberger (1) found that *Nigrospora oryzae* grew well on a wide variety of media, including repurified cellulose, and concluded that such adaptability indicated that the fungus was saprophytic in nature. Much better response of the fungus on nitrogenous media than on media containing starch was obtained by Durrell (2). In this connection he observed that the embryo of the maize kernel was invaded before the starchy endosperm.

An understanding of the relationship between the chemical and physical characteristics of the cob and incidence of infection would help to clarify our knowledge of the disease. Studies were arranged, therefore, to show why the fungus attacked poorly matured cobs more frequently than well-matured ones.

DESCRIPTION AND SOURCES OF COBS AND OTHER MAIZE TISSUES EXAMINED IN THIS STUDY

The inbreds, single crosses, and open-pollinated corn from which cobs were harvested for testing throughout this investigation were chosen on the basis of the maturity of the ears. All the corn was grown at Ames, Iowa, in 1938, 1939, and 1940. An Indiana inbred line, 38–11, was used in certain tests because, due to late-date planting, the ears were harvested in a very immature condition. The inbreds designated as 2, 90, 108, 113, and 116 are experimental lines carried by C. S. Reddy of the Iowa Agricultural Experiment Station. Well-matured ears were produced by 90 and 116. Poorly matured ears were produced by 2 and 113. Inbred 113 was very susceptible to Nigrospora infection in the field; 64 of 68 ears examined were infected in 1940. In the case of inbred 108, 2 ears usually were pro-

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duced to a stalk, the primary ear well-matured and the secondary poorly matured. Os426, L289, and Mc401 are Iowa inbred lines, all of which produced poorly matured ears. B2, an Indiana inbred line, and Pr, an inbred developed by E. W. Lindstrom of the Iowa Agricultural Experiment Station produced ears intermediate in maturity. Single crosses carried under my own experimental numbers, 4228, 1531, and 2317, produced well-matured ears. Cobs of small secondary ears, which were poorly matured, also were used in certain tests.

PHYSICAL DIFFERENCES BETWEEN WELL-MATURED AND POORLY MATURED COBS

It is known that well-matured cobs are harder and woodier than poorly matured ones. It was found that the latter contained more water-soluble substances than did the former. For example, meal made from poorly matured cobs of inbred 38–11 contained 13.0 per cent of water-soluble solids, in contrast to 3.8 per cent in meal from well-matured cobs of the single cross 4228.

Poorly matured cobs were found to have a greater water-absorbing capacity than well-matured cobs. Dry cobs, selected on the basis of maturity, were weighed, soaked in water for 12 hours, wiped with a damp towel, and reweighed. In the first experiment the cobs were selected from field corn on appearance alone. The ratio of weight of water absorbed to cob weight ranged from 1.5 to 6.0 with an average of 2.8 for 18 poorly matured cobs, and from 0.9 to 1.9, with an average of 1.5 for 18 well-matured cobs. In a second experiment cobs from selected inbreds were used. Of 17 poorly matured cobs the ratio of weight of water absorbed to cob weight ranged from 1.6 to 2.6 with an average of 2.1, and a range of 1.1 to 1.9 with an average of 1.5 was obtained for 17 well-matured cobs.

RELATION OF pH TO GROWTH OF NIGROSPORA ORYZAE ON THE COB

Reddy (4) found that cobs of inbreds in which Nigrospora dry rot was prevalent had a higher pH than cobs of inbreds with a low incidence of the disease. In connection with studies[3] on the pathogenicity of this fungus, cob pH was found to be high in immature ears of all inbreds, becoming lower during the season in cobs that matured well but remaining high in poorly matured cobs. To study the effect of the pH of the cob on the growth of the fungus, experiments were arranged in which *Nigrospora oryzae* was grown on well-matured and poorly matured cobs at different pH levels.

Two-gram portions of meal from cobs of the well-matured single cross 4228 or of the poorly matured inbred 113 were added to 50-ml. portions of 2 per cent agar. To some of these portions were added small amounts of dextrose or peptone or both, as shown in table 1. The normal pH of the cobs of single cross 4228 was 4.6, and that of inbred 113, 5.2. These pH levels were interchanged by the addition of lactic acid or sodium hydroxide. The

[3] To be published later.

media thus prepared were autoclaved. *Nigrospora oryzae* was transferred to the media, and the cultures incubated at room temperature for 4 days. Data from 4 replications of this experiment are summarized in table 1.

The experiments with cob-meal agar were checked by parallel experiments with unground cob sections. The results were fully in agreement with those obtained with cob meal. Artificial change in pH had no appreciable effect on the growth of *Nigrospora oryzae* on maize cob. These results point to the conclusion that pH is related to cob maturity, but in itself has no great effect on the growth of the fungus.

CHEMICAL ANALYSES OF COBS OF DIFFERENT MATURITIES

Analyses of well-matured and poorly matured cobs were made to determine differences in composition between the two groups. Apparently sound cobs were selected in all but one case. Samples were arranged in pairs so as to compare well-matured with poorly matured cobs. Analyses were made

TABLE 1.—*Growth of Nigrospora oryzae on media containing meal from poorly matured and well-matured maize cobs, at two different pH values, with and without added nutrients*

Source of cob meal	pH	Response of fungus			
		No nutrient added	0.06 g. dextrose in 50 ml.	0.03 g. peptone in 50 ml.	0.06 g. dextrose 0.03 g. peptone in 50 ml.
Single cross 4228	4.6[a]	+[b]	+	+	++
	5.2	+	+	+	++
Inbred 113	5.2	++	++++	+++	+++++
	4.6	++	++++	+++	+++++

[a] The normal pH is recorded first and the adjusted pH second in each case.
[b] Growth of the fungus is rated on an arbitrary scale on which + represents the poorest and +++++ the best growth obtained.

of oven-dried material according to the methods described by Loomis and Shull (3). The results are reported in table 2. The percentages recorded for each constituent of the cobs are the average of duplicate samples from two separate tests.

The first pair of samples consisted of cobs from primary and secondary ears of inbred 108, harvested in 1938. These two samples are designated as 108p and 108s, respectively. Primary ears of this inbred matured well in the field and were not observed infected by *Nigrospora oryzae*. In years favorable to the growth of the inbred a secondary ear was produced on almost every stalk. These secondary ears were poorly matured, and in 1938 approximately 40 per cent of them were infected by *N. oryzae*.

The second pair of samples in the analyses consisted of meal of well-matured primary ears and of poorly developed secondary ears from the same stalks, the weight of the secondary ears in each case being less than 0.3 that of the respective primary ear. These samples are designated by P (primary) and S.3 (secondary), respectively. Such small secondary ears were frequently infected in the field. This material was harvested in 1938.

The third pair of samples consisted of cobs from secondary ears that weighed at least 0.7 as much as the corresponding primary ear, and cobs from the primary ears. These samples are designated by P′ (primary) and S.7 (secondary). Secondary ears of this size were nearly as free of Nigrospora infection as the primary ears. These ears were harvested in 1938.

During a previous study of the disease, C. S. Reddy of the Iowa Agricultural Experiment Station set aside two samples of cob meal, one from cobs of inbreds in which Nigrospora incidence was low, and the other from inbreds in which infection was frequent. These were included in the analyses and designated as OR and OS, respectively.

TABLE 2.—*Chemical analyses of cob meal from well-matured and poorly matured cobs*

Sample	Amount of each constituent calculated as percentage dry weight of sample						
	Reducing sugar	Non-reducing sugar (as glucose)	Total sugar (as glucose)	Nitrogen	Soluble in 80 per cent alcohol	Residue HCl digestion	Lignin
108p	0.15	0.14	0.29	0.030	3.66	41.5	13.7
108s	0.48	0.43	0.91	0.044	5.89	38.3	12.0
P	0.39	0.14	0.53	0.022	4.07	42.5	14.3
S.3	1.06	1.18	2.26	0.037	8.04	39.8	12.9
P′	0.20	0.13	0.33	0.020	3.55	41.4	12.8
S.7	0.27	0.09	0.36	0.027	4.20	41.5	12.5
OR	0.76	0.42	1.18	0.021	5.03	41.7	17.8
OS	1.03	2.69	3.72	0.039	7.95	38.0	15.5
90	0.22	0.09	0.31	0.028	2.83	40.3	12.3
113	tr.	0.0	tr.	0.025	5.64	43.3	10.0
Diseased	0.88	0.27	1.15	0.092	8.65	36.7	11.9
Not diseased	0.73	0.14	0.87	0.036	4.76	42.6	13.1

The fifth pair of samples in the analyses were from cobs of the inbred 90, well-matured, with no Nigrospora infection in 1938, and from cobs of inbred 113, poorly matured, and with approximately 70 per cent of the ears infected with Nigrospora in 1938. The samples are designated as 90 and 113.

In addition to the samples paired on the basis of maturity, an analysis was made of material from infected cobs separated into portions retted by the fungus and portions in which the fungus had not developed extensively. These two samples are designated diseased and not-diseased.

The data summarized in table 2 indicated that the poorly matured cobs contained more available food than the well-matured ones. In general there was more lignin in well-matured cobs than in poorly matured ones, but the difference was not great. The nitrogen percentage appeared to be somewhat higher in poorly matured than in well-matured cobs.

A physical examination of tissues retted by the fungus showed that much of the substance of the retted part was destroyed. Analyses of retted and nonretted portions of infected cob, summarized in table 2, showed that the

percentages of residue from the HCl digestion and the lignin percentages were about equal for each sample. Further study is necessary to understand the effect of the fungus on the insoluble portion of the cobs.

The low sugar content of cob meal from inbred 113 was unlike that of the other susceptible tissues. This inbred died very early in the field, with ears in a poor state of maturity. The cobs used in the test were harvested late in the fall. It appeared likely that the sugar content of these cobs might have been exhausted by bacterial growth or by other agencies. Collections of ears of inbred 113 were made in the fall of 1939, harvesting the first ears soon after the plants had commenced to die. Two subsequent harvests were made at weekly intervals, and a final harvest was made late in the fall. Cobs from these successive harvests were analyzed for sugar (Table 3).

TABLE 3.—*Sugar content of cobs of inbred 113 at successive dates following death of the plant (1939)*

Date	Percentage total sugar (as glucose)
September 11	2.58
September 18	1.38
September 26	0.53
October 20	0.58
	0.00
	tr.
	0.81

Each analysis was made on a composite meal sample prepared from 6 or more cobs, except for the late harvested ears, analysis of which was made of four separate cobs. The sugar content in cobs of inbred 113 was relatively high early in the fall but decreased rapidly following death of the plant.

GROWTH OF NIGROSPORA ORYZAE ON SUBSTANCES SIMILAR TO SEVERAL COB CONSTITUENTS

To obtain a clearer picture of the manner in which *Nigrospora oryzae* assimilates different cob constituents, the fungus was grown on media containing substances similar to certain of those found in maize cobs. These substances are listed in order of the growth of the fungus as measured by visual examination: 2 per cent dextrose, 2 per cent sucrose, 1.2 and 0.6 per cent peptone,[4] 2 per cent hemicellulose,[5] 2 per cent xylan[6] (the above in Czapek's nutrient solution[7] with 2 per cent agar), 0.5 per cent pectin in Czapek's nutrient solution, cellulose in the form of filter paper,[8] and lignin,[9]

[4] Bacto-Peptone, Difco Laboratories.

[5] Kindly donated by Dr. N. H. Gross of the Bacteriology Department, Iowa State College. The hemicellulose was prepared from straw.

[6] Obtained from Pfanstiehl Chemical Company.

[7] The Czapek's nutrient solution consisted of the mineral constituents only.

[8] Whatman's No. 30.

[9] This was prepared from cornstalks and kindly donated by Dr. L. K. Arnold of the Chemical Engineering Department, Iowa State College. It was purified according to Dr. Arnold's suggestion, by five alternate treatments with HCl and NaOH.

the last two wet with Czapek's solution. With none of these was growth nearly so vigorous as on cob-meal agar or on potato-dextrose agar, employed as checks. Growth on hemicellulose and xylan appeared to be nearly as good as that on the dextrose, sucrose, and peptone. On pectin, growth was very slow, and even slower but still appreciable on cellulose and lignin.

In a further test, growth of *Nigrospora oryzae* on filter paper wet with water was compared with that on filter paper wet with Czapek's nutrient solution. The fungus was allowed to grow for a month or more. When the papers were examined the fungus had sporulated sufficiently to exhibit a distinct, gray color on paper wet with Czapek's solution; but, on the filter paper wet with water, sporulation was so sparse as to be visible only with the aid of the microscope.

The possibility that traces of soluble nutrients influenced the growth of *Nigrospora oryzae* on lignin, cellulose, and pectin, even though each of these had been treated to remove soluble nutrient substances, would not be eliminated without further investigation. It was of interest that growth of *N. oryzae* on media containing xylan and hemicellulose compared well with that on media containing dextrose. Of especial interest was the vigorous growth of the fungus on cob-meal agar and on potato-dextrose agar as compared with that on the other media tested. Certain constituents present in cobs and in potatoes apparently were lacking in all the other substances tested.

PRELIMINARY TESTS WITH COB CONSTITUENTS FAVORING GROWTH OF NIGROSPORA ORYZAE

A few preliminary tests were made to obtain information concerning the nature of the substance in maize cobs favoring growth of the fungus. That substances in poorly matured cobs favored growth of the fungus, rather than that substances in well-matured cobs interefered with growth of the fungus was ascertained by adding cob meal prepared from well-matured and poorly matured cobs to plain agar. Growth on agar containing meal of well-matured cobs was much better than on agar without cob meal but not nearly so good as on agar containing meal of poorly matured cobs. Substances favoring the growth of the fungus occurred to some extent in well-matured cobs.

The substances favoring growth of *Nigrospora* in cobs were largely water-soluble. Growth of the fungus was extensive on media prepared from cob extracts, but sparse on media prepared from the leached cob meal.

Growth of the fungus was found to be slightly better on media containing cob meal from cobs retted by *Nigrospora oryzae* than on media containing equal amounts of meal prepared from poorly matured cobs. Apparently the growth of *N. oryzae* on maize tissues did not exhaust substances favorable to its own growth, nor were there formed heat-stable staling products unfavorable to the growth of the fungus.

Autoclaving cob meal (in 2 per cent agar agar) for as long as $3\frac{1}{2}$ hours at 15 lb. pressure did not make meal from well-matured cobs more favorable

for growth of the fungus, nor did such autoclaving render meal from poorly matured cobs less favorable.

An experiment was arranged to determine whether the substances favoring growth were of organic nature. Portions of cob extract charred at red heat were added to Czapek's nutrient solution with 2 per cent dextrose. Growth of *Nigrospora oryzae* on this medium was not better than that on the nutrient solution alone, while growth in the presence of uncharred extract was profuse. This experiment did not completely eliminate the possibility that certain minerals might be the influential factors in rendering poorly matured maize cobs liable to Nigrospora infection, for the chemical nature of the minerals may have been altered in the charring process.

GROWTH OF NIGROSPORA ORYZAE ON WATER EXTRACTS OF MAIZE COBS

In recording the growth of the fungus on cob-meal media an arbitrary scale must be used. The area of mycelial growth proved unsatisfactory as a criterion in that it did not distinguish between sparse and heavy growth of similar extent. The use of liquid cultures from which mycelial mats could be weighed offered a more satisfactory method of comparison; therefore, in further work, liquid cultures were employed. Tests parallel to those described in this section, but in which cobs and cob-meal agar were employed confirmed the results obtained with cob-meal extract. The principal purpose of the study on water extracts of cobs was to compare the effects of extracts of poorly matured and well-matured cobs on the growth of the fungus. In doing this, 20-ml. portions of Czapek's nutrient solution were placed in 500-ml. Erlenmeyer flasks. The hydrogen-ion concentration was adjusted in all cases to approximately pH 5.0 with lactic acid to reduce bacterial contamination. After the addition of cob extract, and in some cases dextrose, and autoclaving uniform pieces of Nigrospora culture on nutrient agar were transferred to the media, and the cultures were incubated at room temperature for 4 days. At the end of this time the mycelial mats were washed on tared percale disks and dried. The heavier mats were gelatinous in consistency, and it was necessary to remove most of the water from these mats by placing the percale disks on a series of blotters before transferring to the 100° C. oven for final drying. After drying, the percale disks with the mycelial mats were weighed.

The cob-meal extracts, obtained by placing the cob meal in boiling distilled water, contained the leachate of 1 g. of meal in each milliliter of extract. The extract of inbred 38–11, on which the fungus grew very well, was used as an arbitrary standard in testing the effect of the cob extracts on the growth of the fungus. In a preliminary test various amounts of extract from cob meal of this inbred were added to 20-ml. portions of Czapek's nutrient solution (2 per cent dextrose). Amounts of extract greater than 0.4-ml. did not produce corresponding increases in growth of the fungus. Thereafter, in making comparisons, 0.4-ml. portions of extract were used.

TABLE 4.—*Mycelial weights of Nigrospora oryzae grown in Czapek's nutrient solution with and without extract of well-matured and poorly matured cobs*

Dextrose added	Weight of mycelium in milligrams (average, 7–9 samples)			
	Check	Single cross 4228 (well-matured)	Inbred 108 (well-matured)	Inbred 38–11 (poorly matured)
None	1.9	5.3	17.4	45.5
2 per cent	3.1	37.0	58.0	130.8

In the first series of tests the fungus was grown on Czapek's nutrient solution, on Czapek's nutrient solution with 2 per cent of dextrose, on nutrient solution with cob extracts, and on nutrient solution with cob extracts and 2 per cent of dextrose. The growth of the fungus in response to cob extract of the poorly matured inbred 38–11 was compared with that in response to extracts of the well-matured inbred 108 and single cross 4228 (Table 4). Without cob extract there was no essential difference in growth with and without dextrose. With the addition of cob extract, growth was much better in the solution containing dextrose than in solutions containing no dextrose. The best growth of the fungus occurred with the addition of extract of poorly matured cobs to medium containing dextrose. Apparently dextrose and substances present in the cob extract influenced the growth of the fungus.

Cob-meal extracts of several poorly matured and well-matured inbreds and single crosses were compared in respect to their ability to support the growth of *Nigrospora oryzae* in liquid culture. In this experiment dextrose was added to the media throughout. The average weights of mycelial mats, with data on the incidence of infection of cobs used as the source of the extracts, are recorded in table 5. In all tests the best growth of the fungus occurred in the presence of extract of poorly matured cobs.

TABLE 5.—*Growth of Nigrospora oryzae on Czapek's nutrient solution containing two per cent dextrose with extracts of cobs of different maturities*

Source of cob extract	Nigrospora infection in the field. No. of ears		Maturity	Weight of mycelium in mg. (ave. 6 samples)
	Examined	Infected		
Ind. inbred 38–11	.....	[a]	Poor	140.4
Inbred OS426	53	21	Poor	76.1
Inbred OS420	46	20	Poor	63.2
Inbred 2	38	14	Poor	71.1
Inbred L289	43	19	Poor	73.2
Inbred B2	60	0	Fair	42.3
Inbred Pr	54	0	Fair	38.7
Inbred 116	102	2	Good	27.3
Single cross 1531	56	0	Good	24.9
Single cross 2317	64	0	Good	22.6
Check	.....	.....	.....	1.8

[a] Inbred Ind. 38–11 was harvested in a very immature condition, while the ears were still green. No infection was present in these ears at time of harvest; infection has not been detected in maize tissue in green condition.

In these two series of tests growth of the fungus in liquid culture was more profuse with that without cob extract, but considerably more profuse with extracts of poorly matured than well-matured cobs.

Studies are being continued on the nature of the substances, more abundant in poorly matured than in well-matured cobs, which favor growth of *Nigrospora oryzae*.

SUMMARY

A study was made of physical and chemical characteristics of maize cobs in relation to prevalence of infection by *Nigrospora oryzae*. Poorly matured cobs were found to be more frequently infected than well-matured cobs.

Poorly matured cobs were less woody than well-matured cobs. The water absorbing capacity of poorly matured cobs was greater than that of well-matured cobs.

The pH of the cob was found to be an indication of cob maturity. Poorly matured cobs have a higher pH than well-matured cobs.

Poorly matured cobs contain more water soluble substances than well-matured cobs. Chemical analyses showed that poorly matured cobs contain more available food, especially sugars, and less lignin, than well-matured cobs. The high sugar content of poorly matured cobs may be dissipated if harvest of the ears is delayed.

Nigrospora oryzae grew well on media containing sugars, peptone, xylan, and hemicellulose, but only slight growth was obtained on pectin, cellulose, and lignin. Growth on cob-meal agar was far more vigorous than on any of the simpler nutrients.

A substance or substances, other than simple nutrients, present in cobs favored growth of *Nigrospora oryzae*. The substance or substances were present in greater abundance in poorly matured than in well-matured cobs. The substance or substances were water soluble and heat stable, and appeared to be organic in nature.

A close relationship exists between susceptibility to Nigrospora infection and growth of the fungus in response to cob extracts.

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TWO NEW VIRUSES AFFECTING TOBACCO AND OTHER PLANTS

KENNETH M. SMITH AND ROY MARKHAM

(Accepted for publication June 10, 1943)

INTRODUCTION

In this paper are given short descriptions of two apparently new or undescribed viruses. They are probably of little economic significance but are of considerable interest because both viruses appeared in an unknown manner in plants growing inside the insect-proof glasshouses at Cambridge.

The first virus to be described was isolated in mid-winter from a plant of *Arabis hirsuta,* which had been growing for a number of years in the glasshouse and had been used for stock cultures of cabbage mosaic (*Brassica virus 1,* Smith). The second virus was isolated from a noninoculated tobacco plant in the summer of 1942, also growing in the glasshouse.

ARABIS-MOSAIC VIRUS

Properties of the Virus

Thermal Inactivation Point. The virus is inactivated by a 10-minute exposure to a temperature of 60° C., but not by a similar exposure at 50° C.

Dilution End-point. In crude sap expressed from infected White Burley tobacco plants, the virus is infective at a dilution of 1:100 but not at 1:1,000.

Resistance to Aging. The longevity *in vitro* of the virus is between 48 and 72 hours at room temperature.

Transmission. The virus is sap-inoculable, but is not very infectious; it is more difficult to transmit during hot weather when symptoms tend to be masked. All attempts to transmit the virus by means of the aphids *Myzus persicae* and *Aphis fabae* have failed.

Host Range and Symptomatology

Arabis hirsuta. The symptoms on this plant consist mostly of ring and line patterns of dark-green on a lighter background, together with a certain amount of mottling. No necrosis has been observed. Incidentally, it may be mentioned here that great difficulty has been experienced in transmitting the virus to fresh *Arabis* plants.

Nicotiana tabacum var. White Burley. As a rule there are no definite local lesions formed on the inoculated leaf, although slight chlorotic spots have occasionally been observed. Systemic infection takes the form of scattered chlorotic rings, which may be concentric, on the outer leaves. The central shoot then develops a preliminary pallor or chlorosis, followed by a very characteristic splitting of the leaves, and one side or both sides of the lamina may be stripped away. These leaves invariably become necrotic. This peculiar stripping or shredding of the central leaves, together with a

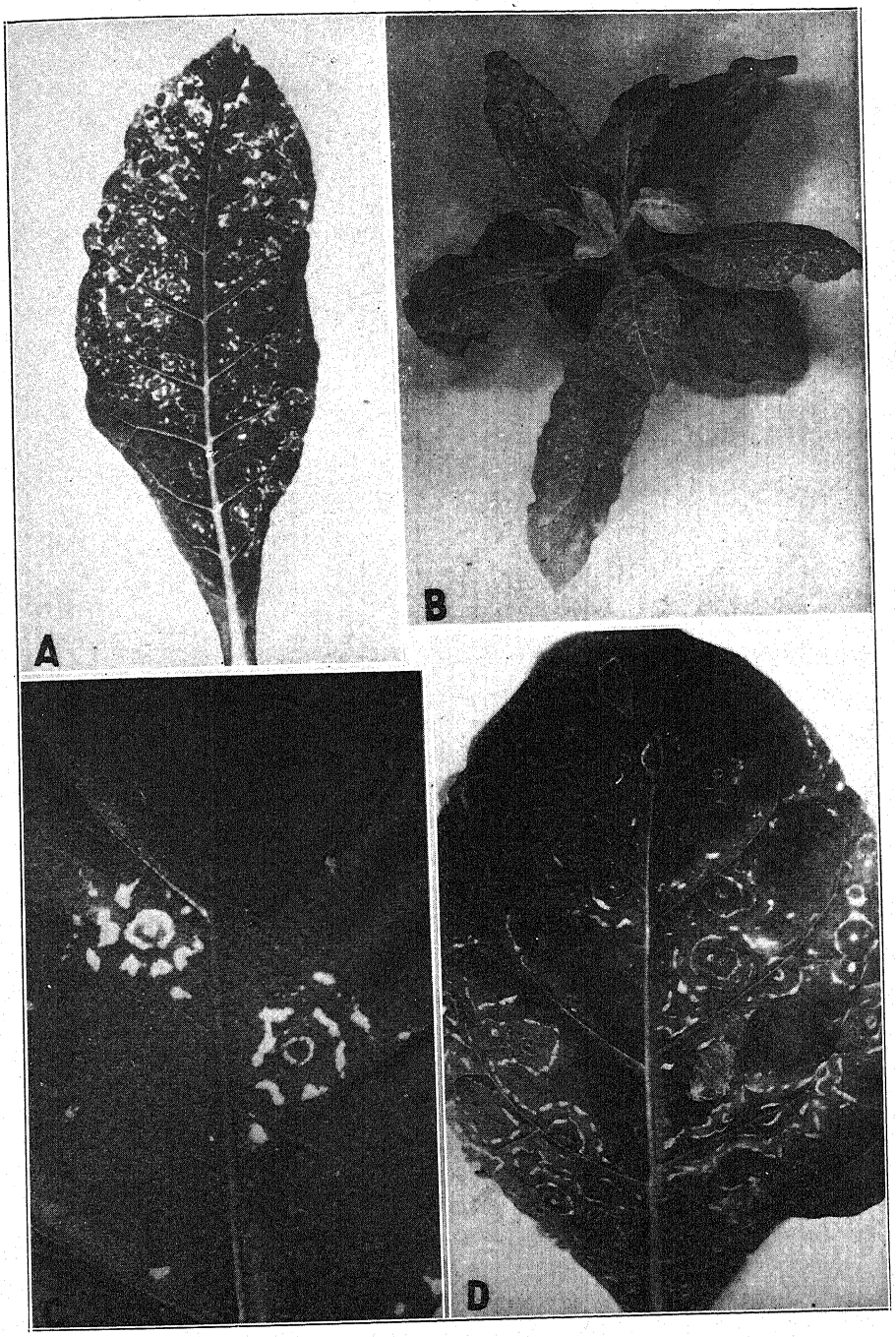


FIG. 1. A. Ring and line patterns produced by the *Arabis* virus on White Burley tobacco. B. Tobacco, White Burley, systemically infected with the *Arabis* virus. Note the characteristic splitting and puckering of the central leaves. C. Incomplete or "broken" rings on White Burley tobacco. D. Systemic symptoms of "broken ring spot" on White Burley tobacco.

reddish necrosis, is the most characteristic symptom of the whole disease (Fig. 1, B). As the central leaves develop, the necrosis increases and the tips pucker up and bend downwards and inwards. At the same time necrotic spots develop on the rest of the plant, and these frequently tend to become ring-like (Fig. 1, A). At this stage the disease is very severe and is particularly so during the winter and at low temperatures.

The incubation period of the virus in the tobacco plant varies from 10 to 21 days, according to the temperature, but may be much longer in very hot weather, when the symptoms are sometimes entirely masked. After a time the plant grows away from the more acute symptoms and looks almost normal, except for a slight mottle and a tendency for the leaves to be dark-green.

Nicotiana glutinosa. On this plant the symptoms are similar to those on the tobacco plant but may be more severe, and the disease is frequently lethal.

Solanum nodiflorum. Chlorotic rings have been observed on the inoculated leaves of this species, but systemic infection does not appear to develop.

Cucumber. The cucumber plant is susceptible to infection with the *Arabis* virus, though the incubation period may be very long, sometimes more than 3 weeks. The symptoms consist of a fairly typical mosaic mottle not unlike that due to cucumber mosaic itself. There are no local lesions formed.

Phaseolus vulgaris, French Bean, var. Canadian Wonder. No local lesions are formed on the inoculated bean leaves, and this is a point of difference from the ring-spot virus, which is the second of the two viruses described in this paper. Systemic infection first develops as small yellow flecks on the young leaves, about a week or 10 days after inoculation. There are, sometimes, raised blisters with the intervening areas pale. The yellow flecks become larger and produce a fairly bright mosaic mottle (Fig. 2, A). This usually is followed by a severe necrosis resulting in the death of the growing points and youngest leaves.

Possible Relationships of the Arabis Virus

Since the virus was first obtained from an *Arabis* plant, which was systematically infected with cabbage mosaic (*Brassica virus 1,* Smith), it seemed possible that the *Brassica* virus might have mutated to give rise to the second virus. Cross-immunity tests, however, do not support this theory. These tests were carried out on the tobacco plant and, since *Brassica virus 1* does not become systemic in tobacco, inoculations were made of *Brassica virus 1* into tobacco plants systemically infected with the *Arabis* virus. The reddish local lesions, characteristic of *Brassica virus 1* on the tobacco plant, duly developed in spite of the presence of the *Arabis* virus.

The fact that the virus will infect the cucumber plant, and also some of its physical properties suggest that it might be a derivative from one or other of the strains of cucumber-mosaic virus propagated at the virus research

station. Again, however, experimental evidence does not bear this out. Tobacco plants systemically infected with the *Arabis* virus were reinoculated with a strain of cucumber-mosaic virus. A strain that gives a bright-yellow mottle was used, so that there could be no doubt whether infection

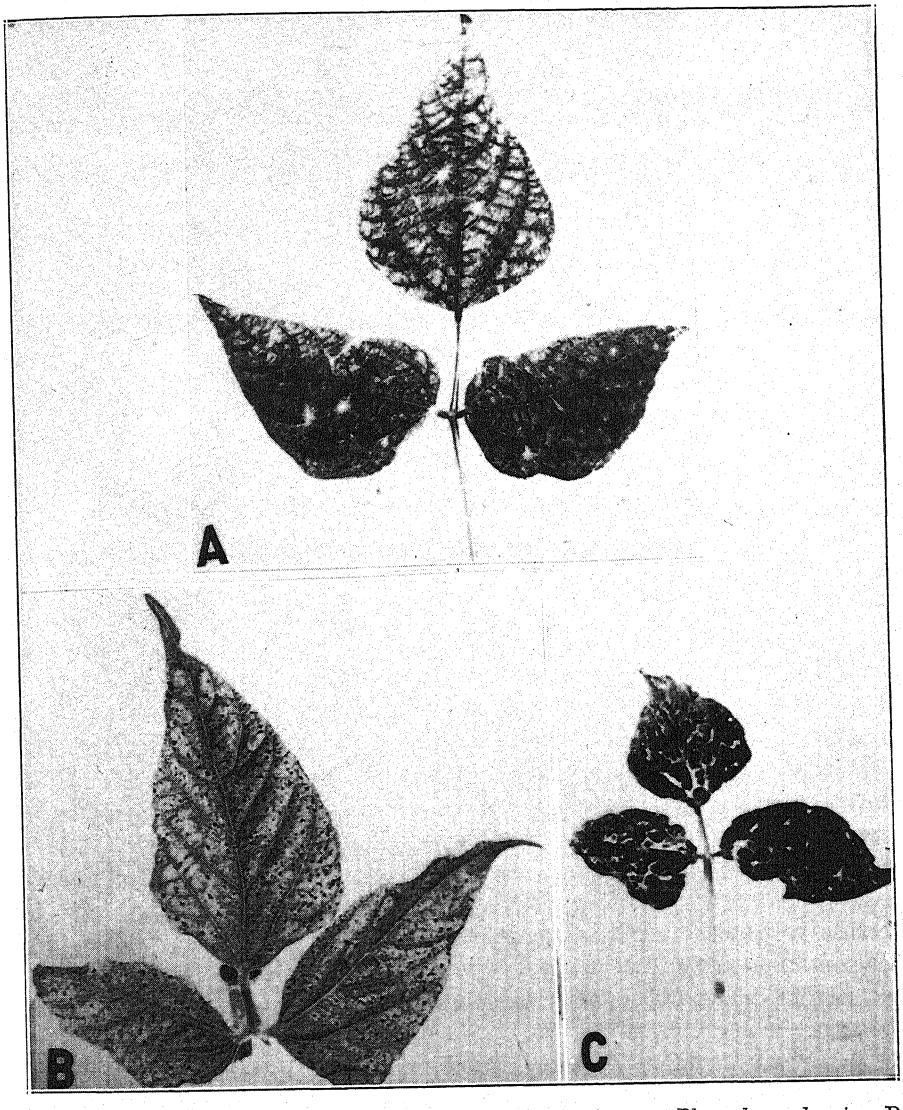


FIG. 2. A. Mosaic mottle produced by the *Arabis* virus on *Phaseolus vulgaris*. B. Numerous small necrotic spots formed on *P. vulgaris* by the broken ring-spot virus. C. Blister mosaic on *P. vulgaris* caused by infection with broken ring-spot virus.

took place. The brilliant mosaic characteristic of this virus developed normally, even though the plants were systemically infected with the *Arabis* virus. Certain other facts militate against the theory that the *Arabis* virus may be a strain of cucumber-mosaic virus. There is the fact that aphids

apparently do not transmit it, and there is its limited host range. For example, it does not infect *Datura stramonium*, a plant that is extremely susceptible to the cucumber-mosaic virus.

Tobacco Broken Ring-spot Virus

A single necrotic ring was observed in a noninoculated tobacco plant, var. White Burley, in the glasshouse. This was cut out and reinoculated to a further series of tobacco plants of the same variety. After a time the plants developed a fairly typical ring-spot disease. It is somewhat difficult to find a new and descriptive name for this virus, since there are already 5 or 6 quite distinct viruses that cause a more or less similar ring spot in tobacco. Recently, Johnson and Fulton[1] have described yet another tobacco ring spot, which they have called "tobacco broad ring spot"; and, since the rings caused by the present virus are frequently incomplete, a suitable name seems to be "tobacco broken ring spot" (Fig. 1, C).

Properties of the Virus

Thermal Inactivation Point. The virus is inactivated by a 10-minute exposure to a temperature of 60° C.

Dilution End-point. Using crude expressed sap, clarified by spinning, positive infections were obtained at a dilution of 1:100 but not at 1:1000.

Resistance to Aging. The virus retains infectivity in extracted sap at room temperatures for about 6 days.

Transmission. The virus is fairly easily transmitted by sap inoculation, but no insect vector so far has been discovered.

Host Range and Symptomatology

Nicotiana tabacum, var. White Burley. Local lesions may develop, but they do not always occur; when they do appear they take the form of a cluster of small single rings on the inoculated leaf. Later, isolated single rings or ring and line patterns develop on the noninoculated leaves. These increase in number and coalesce, so that the leaves eventually become covered with the characteristic pattern (Fig. 1, D). As a rule there is little necrosis or stunting, such as occurs with the tobacco ring spot of Wingard[2] (*Nicotiana virus 12*), in the same variety of tobacco. Local lesions, if present, develop about 5 days after inoculation, followed about a week later by the systemic symptoms.

Nicotiana tabacum, Turkish, var. Kawala. Symptoms in this variety resemble those on White Burley, except that local lesions seem always to form on the inoculated leaves. These lesions usually are single rings, and bear a superficial resemblance to the rings produced by potato virus X on Turkish tobacco.

[1] Johnson, James, and R. W. Fulton. The broad ring-spot virus. Phytopath. 32: 605–612. 1942.

[2] Wingard, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. [U. S.] 37: 127–153. 1928.

Nicotiana glutinosa. This species appears to be rather resistant to the ring-spot virus; necrotic spots develop, but the disease is not severe and the plant rapidly outgrows the symptoms.

Phaseolus vulgaris, French Bean, var. Canadian Wonder. The reactions of the bean to the ring-spot virus are characteristic and serve to differentiate the ring-spot and *Arabis* viruses, which have some symptoms in common. Very pale chlorotic areas develop on the inoculated leaves within 5 days of inoculation. These are followed a few days later by the systemic symptoms, which take the form of large numbers of minute necrotic spots on the youngest leaves (Fig. 2, B). Frequently, these leaves are killed by the necrosis. Later, a mosaic mottle may develop in which blisters of green tissue stand out from between the necrotic veins, giving the leaves a puckered appearance (Fig. 2, C).

Cucumber. The ring-spot virus produces local lesions on the inoculated leaves of cucumber, and this serves further to distinguish it from the *Arabis* virus. The lesions are white and circular and appear about 5–7 days after inoculation. Systemic symptoms consist of a rather diffuse mottle, less pronounced than that caused by the *Arabis* virus.

SUMMARY AND CONCLUSIONS

Two new viruses affecting tobacco and other plants are described. One was first observed in a plant of *Arabis hirsuta* and the other in tobacco. Both viruses produce diseases of the ring-spot type in tobacco, but the virus from *Arabis* can be distinguished easily by the fact that it induces a characteristic curling and shredding of the central leaves.

The main points of interest concerning these two viruses are their origin and method of spread. Both viruses are new and both appeared in plants that were growing inside the insect-proof glasshouse. Moreover, the disease caused in the *Arabis* plant first appeared in mid-winter. The appearance of these viruses certainly needs some explanation, since they are very uninfectious and no insect vector has been discovered. In consequence, there is no information on their natural mode of spread. Indeed, the viruses would have been lost with the death of the original plants in which each appeared had it not been that they were carefully propagated by mechanical methods of inoculation.

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POLLEN AS A SOURCE OF WALNUT BACTERIAL BLIGHT INFECTION

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The severity of walnut bacterial blight (caused by *Phytomonas juglandis*) in California has frequently been ascribed to numerous over-wintering cankers (2, 3, 4). In Oregon, however, the disease has been found to overwinter in diseased buds (leaf and catkin buds) (1). A review of the literature pertaining to the earlier work on walnut blight (3) indicates that this disease was observed on leaf and catkin buds in California without any special significance being attached thereto. In severe outbreaks of the disease, cankers on young shoots were so numerous that they overshadowed the less conspicuous leaf and catkin buds.

Recently, in California, walnut blight was observed in epidemic form, with but few cankers in evidence. The incidence of disease could not be ascribed to such a small amount of inoculum. Examination revealed the presence of the disease (Fig. 1) in dormant catkin and leaf buds and also the presence of blight bacteria on the pollen of diseased catkins.

In a series of experiments, catkins were collected from trees known to have been heavily blighted the previous summer and, after examination under a dissecting microscope, were cultured individually for blight bacteria. Results showed that of the catkins collected on August 20, 1942, 16 per cent yielded live and virulent cultures of *Phytomonas juglandis;* on September 15, 1942, there was 20 per cent; on October 16, 1942, 15 per cent; on November 12, 1942, 30 per cent; on December 23, 1942, 26 per cent; on January 18, 1943, 18 per cent; and on February 20, 1943, 27 per cent.

Of the blighted leaf buds found on the same trees, from 10 to 26 per cent contained living blight bacteria when cultured from August to February at monthly intervals.

Observations on catkins and leaf buds, beginning in March, in the two previously mentioned localities in California, showed an abundance of blighted catkins in all stages of development (Fig. 2). Catkins occupy a strategic position in relation to subsequent infection of foliage and pistillate flowers. Catkins are highly susceptible organs present on the twigs all year, and are subject to infections all year around whenever conditions favor spread of blight. Dormant catkins (or catkin buds) provide a perennial source of water-borne infection, and when mature may shed contaminated pollen, thus providing for aerial dissemination of the blight organism. Healthy catkins may be invaded and killed whenever conditions favor the spread of the disease. Frequently, catkins are partially infected and will shed pollen from the remaining healthy florets. Pollen from the florets on diseased catkins is readily contaminated and can be broadcast alone or in combination with healthy pollen for a considerable distance, causing infec-

tion whenever environmental conditions are favorable for the development of the blight bacterium.

That pollen from blighted catkins may be contaminated by the blight bacteria was shown in a small scale experiment by C. O. Smith (3). How-



FIG. 1. Multiple leaf-bud infections by *Phytomonas juglandis* on Franquette (left) and Payne (right) varieties of walnut. Note the diseased catkin A on the center twig, which was photographed September 3, 1943.

ever, the importance of this aspect of the disease was not stressed, and no experiments were conducted to ascertain its relation to the epidemiology of walnut blight.



FIG. 2. Catkin blight. Note strategic position of the catkins, close to young growth that will bear pistillate flowers.

The writer studied this aspect of the disease during the spring and summer of 1943. Altogether, 150 diseased catkins were cultured directly in the field by shaking the pollen on nutrient agar plates, using three plates for each diseased catkin. Numerous colonies of *Phytomonas juglandis* developed on all plates when incubated at 28° C. The pathogenicity of the cultures was confirmed by inoculation tests on immature nuts in May and June.



FIG. 3. Walnut blight lesions produced by dusting the leaf with contaminated pollen. Portion of walnut leaf greatly enlarged showing blight lesions produced by dusting with contaminated pollen. Pollen grains shown at A.

Contaminated pollen was mixed with known healthy pollen and this pollen mixture was then tested to determine whether it could induce the disease in pistillate flowers and on walnut leaves. The technique employed by Wood (5) in his walnut pollination tests was adapted for the above-mentioned experiment. The ventral side of leaves was dusted in the leaf experiments. Treated parts were protected with paper bags against contamination. As a check, pollen from healthy catkins was employed in otherwise similar tests. Of 50 pistillate flowers pollinated from healthy catkins,

45 set nuts and no blight infection occurred in these nor in the 5 nuts that dropped. Of 50 pistillate flowers pollinated with contaminated pollen, only 35 set nuts and 20 of these were infected as were also 10 of the 15 nuts that dropped. In all blighted nuts there was only apical type of blight.

In another experiment, leaves were dusted with pollen and bagged. Ten leaves were dusted with pollen from infected catkins and 10 with pollen from healthy catkins. The check leaves were covered with bags, but otherwise not treated. After 1 month no infection had developed in the checks and the leaves dusted with pollen from healthy catkins, while 7 of 10 leaves treated with contaminated pollen bore definite blight lesions (Fig. 3). These lesions yielded cultures of blight organism.

The above-mentioned experiments demonstrate the fact that contaminated pollen may disseminate walnut blight under favorable environmental conditions. Therefore, it is likely that blight infection of nuts may be caused not only by bacteria carried in a film of water during a rainy period from infected leaf buds and catkins, but, also, by contaminated pollen from a partially diseased catkin in which, in the process of natural pollination, is deposited on the stigmas of the pistillate flowers.

The blight organism grows very luxuriantly on agar media containing no other nutrients than walnut pollen. When a culture on this medium is dried completely and held in this condition for several months in the laboratory, it may be revived by plunging a piece of it into a liquid medium.

The blight organism was recovered from dry pollen 4 months after being stored in the vial under laboratory conditions.

The importance of contaminated pollen, together with diseased leaf and catkin buds, may be visualized from Wood's statement (5) that "upon some of the larger Placentia trees as many as 10,000 catkins were counted during a season," and "it was found that single catkins produced from 1,000,000 to 4,000,000 pollen grains."

The prevalence of catkin infection, the presence of viable blight organisms on pollen from infected catkins and the high probability that considerable nut infection may come from this source, emphasizes the importance of infected catkins and contaminated pollen in the overwintering and dissemination of the walnut blight organism.

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A VIRUS DISEASE OF LOVAGE (LIGUSTICUM SCOTICUM)

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(Accepted for publication June 10, 1943)

INTRODUCTION

During the summer of 1940 the attention of one of us (K. M. S.) was drawn to a plant of lovage (*Ligusticum scoticum* L.) growing in a private garden. The leaves were mottled with patches of light and dark green and showed a typical mosaic disease. Inoculation from this plant to tobacco produced a very severe necrotic disease which invariably killed the latter plant. Since the symptoms produced in tobacco were unfamiliar, an investigation of the virus was undertaken and the results are given here. They show that the lovage mosaic was due to a hitherto undescribed virus.

No attempt has been made to undertake a systematic examination of the complete host range but so far as the inoculation experiments go they suggest that the host range of the virus is not very restricted.

HOST RANGE AND SYMPTOMATOLOGY

Umbelliferae

Ligusticum scoticum L. (*Lovage*). The original infected plant of lovage was a bush of considerable size being several years old. The plant was rather stunted in comparison with healthy plants of the same age and the leaves showed a bold somewhat streaky mottle (Fig. 1, A). There was little necrosis.

We found great difficulty in infecting healthy lovage seedlings with the virus, and only succeeded with 1 plant out of about 100 inoculations. As the attempted infection of lovage seedlings had been made from infected tobacco plants, there was the possibility that the difficulty was due to low virus concentration in the tobacco plants relative to lovage. To test this the following experiment was performed. Twenty tobacco plants, var. White Burley, were inoculated from the original infected lovage plant and 20 similar tobacco plants were inoculated from an infected tobacco plant. In both cases 100 per cent infection was obtained, and there seemed no difference in the infective power of the tobacco and lovage sap.

Daucus carota (*Carrot*) *and Apium graveolens* (*Celery*). Attempts to infect these two plants were unsuccessful.

Solanaceae

Nicotiana tabacum var. White Burley. The tobacco plant is extremely susceptible to infection with the lovage virus, and makes a good indicator or test plant. Local lesions are produced about 7 days after inoculation, but they vary considerably in appearance according to conditions of light and temperature. In one case they take the form of small, dark-red, necrotic

circles with lighter green interiors, and in the other case they appear as large chlorotic circles (Fig. 2, A). Systemic symptoms take about 17 days to develop, and they consist of a very severe necrosis of the veins and midrib (Fig. 2, B), which rapidly spreads and causes the death of the plant. At

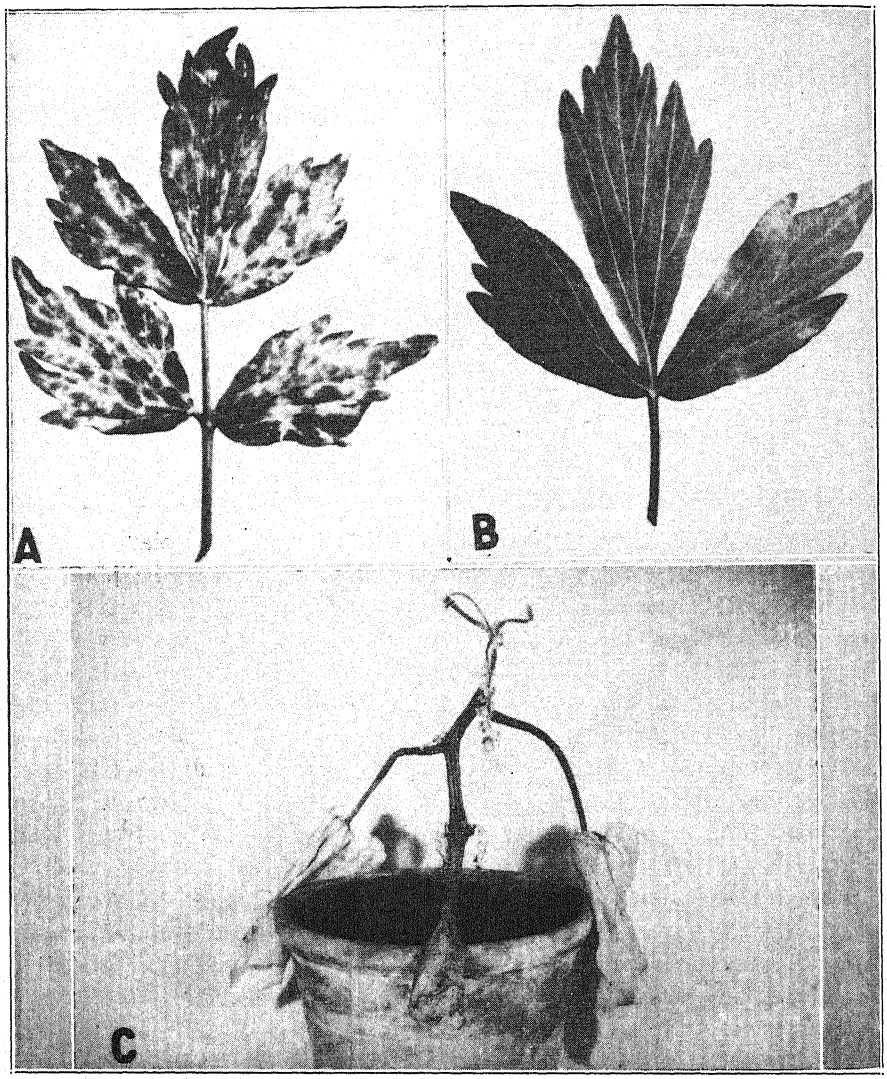


FIG. 1. A. Leaf of lovage plant, showing mosaic mottle. B. Leaf of healthy lovage plant. C. Cucumber plant infected with the lovage virus showing systemic necrosis.

high temperatures the symptom picture may be rather different. Under these conditions there are few local symptoms and the first indication of infection is the appearance of systemic necrosis, which spreads down the petiole of the inoculated leaf and thence reaches the stem. Here it spreads

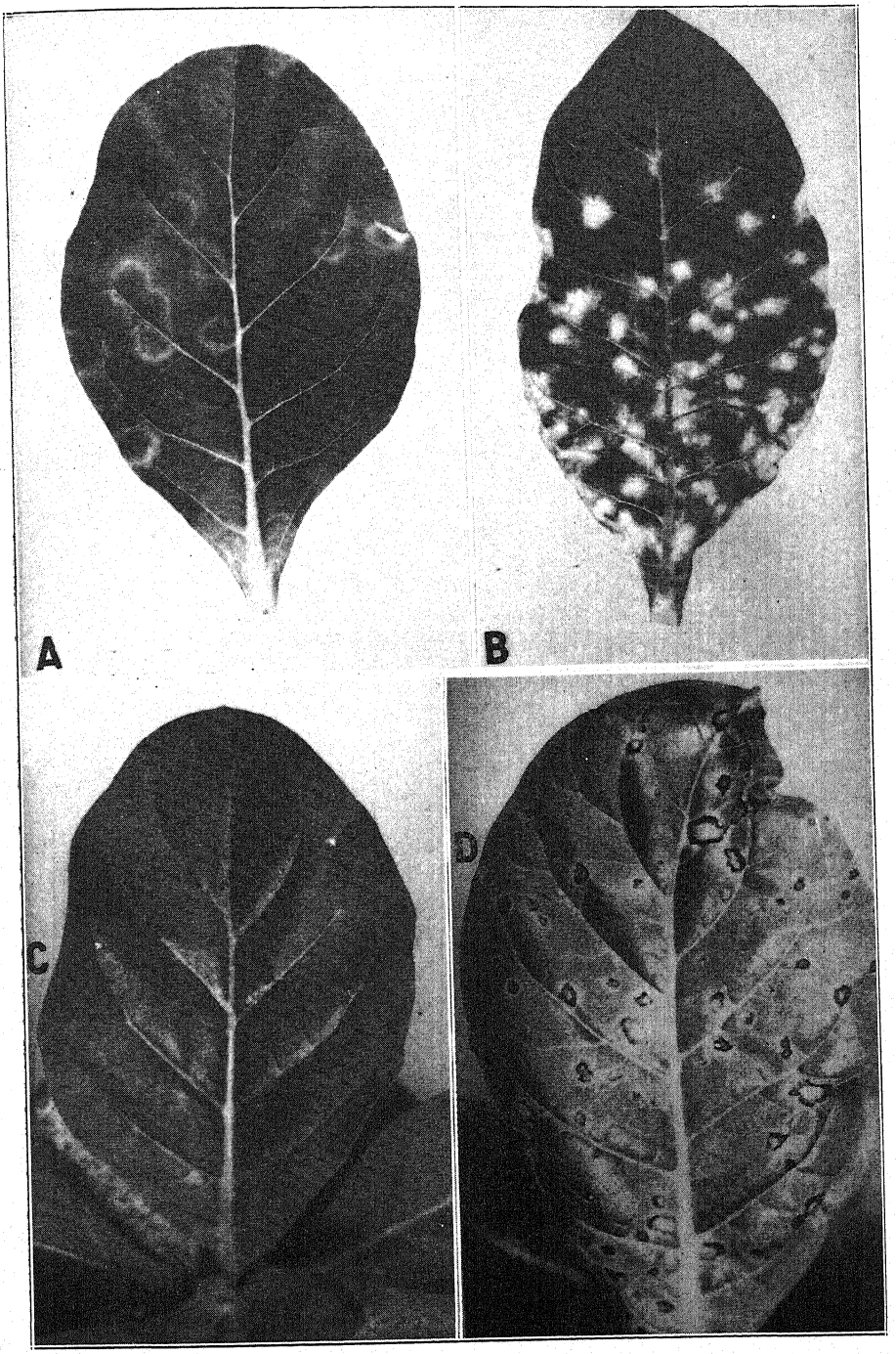


FIG. 2. A. Chlorotic circles on inoculated leaf of White Burley tobacco. B. Systemic infection of White Burley tobacco by the lovage virus. C. Local lesions on *Nicotiana sylvestris*. D. Systemic infection of *N. sylvestris* by the lovage virus.

slowly, both up and down, cutting off the supply of water and food materials so that the plant wilts and dies.

Nicotiana tabacum Turkish, var. Kawala. In this variety of tobacco the leaf symptoms are less pronounced, but, instead, a severe necrosis of the main stem and midribs usually is produced.

Nicotiana glutinosa. Rather faint local lesions are sometimes formed on this species, followed by a faint mottle and some necrosis.

Nicotiana sylvestris. Very distinct and characteristic local lesions develop on this species about 5 days after inoculation (Fig. 2, C). They appear first as shiny glassy spots without a well-defined or necrotic edge. Later, they develop a coppery ring, while still retaining their glassy center. This plant should prove suitable for quantitative studies on the virus. Systemic symptoms develop about a week later and take the form of lesions that are very similar to those formed on the site of inoculation, except that they lack the preliminary glassy stage (Fig. 2, D).

Nicotiana langsdorffii, N. rustica and Capsicum annuum. Local lesions without systemic infection develop on these 3 species.

Lycopersicum esculentum, Tomato, var. Kondine Red. The tomato plant is susceptible to infection, but develops no symptoms and is, in fact, a perfect symptomless carrier of the virus. Infection in other plants is easily obtained by inoculation from such carrier tomatoes.

Datura stramonium. This plant reacts with a faint mottle, there is no necrosis.

Solanum tuberosum. The potato plant appears to be immune from infection.

Leguminosae

Phaseolus vulgaris var. Canadian Wonder. The bean is susceptible to infection with the lovage virus, yellow chlorotic spots developing on the inoculated leaves after 3 or 4 days. Signs of systemic infection develop about a week later as a slight veinal necrosis of the younger leaves, portions of the veins being picked out in yellow. This is followed by a gross systemic necrosis of the youngest leaves, which involves the death of the growing point.

Pisum sativum (Garden Pea). A number of different varieties of garden pea were inoculated, but infection was secured only on the variety English Wonder. In this variety there developed a reddish necrosis of the veins of the youngest leaves and this was followed by the death of the growing point.

Cucurbitaceae

Ridge Cucumber. A slight mosaic mottle develops on the leaves of cucumber plants, and this is followed by a severe necrosis that kills the growing point. The disease produced on cucumber by this virus almost invariably results in the death of the plant (Fig. 1, C).

An experiment was carried out to investigate whether there was any cross immunity between the lovage virus and cucumber-mosaic virus. Six

tobacco plants, systemically infected with a yellow strain of cucumber-mosaic virus, and 6 similarly infected with the type virus were inoculated with the lovage virus. All 12 tobacco plants developed the necrotic symptoms characteristic of infection with the lovage virus. There seems, therefore, to be no relationship between this virus and that of cucumber mosaic.

Malvaceae

Lavatera trimestris (*Garden Mallow*). The cultivated mallow is susceptible and develops a faint yellow mosaic. There is no necrosis.

Cruciferae

Arabis hirsuta (*Rock Cress*). This plant is susceptible, but the symptoms are rather indeterminate; there may be a tendency to ring formation. The virus was recovered from infected *Arabis* plants by inoculation back to other susceptible species.

Some Properties of the Virus

Transmission. With the exception of the lovage plant itself, the virus is easily transmitted by sap inoculation to most of its host plants, but no insect vector is known. Attempts to transmit the virus by means of the aphis, *Myzus persicae,* were unsuccessful.

Thermal Inactivation Point. The virus was inactivated by a 10-minute exposure to a temperature of 60° C., but not to a temperature of 55° C.

Dilution End-point. Using crude extracted sap from infected White Burley tobacco, clarified by spinning, some positive infections were obtained at dilutions of 1 : 100 but not at 1 : 1000. The concentration of the virus in its host plant, therefore, seems to be very low.

Longevity in Vitro. The virus remains infective for about a week in crude expressed sap at room temperature.

DISCUSSION

From a consideration of the symptomatology of the lovage virus, there is little doubt that it has not been previously described. There are one or two points of interest in regard to the virus that might be emphasized here. Its sudden appearance in an isolated plant from which it does not seem to have spread in 3 years, and the fact that it does not appear to be insect-transmitted are rather unusual. It might perhaps be expected that adjacent celery plants, which, after all, are closely related botanically to lovage, would have become infected, but attempts to transmit the virus to celery artificially have proved unsuccessful. Another point of interest is the comparatively high death rate caused by the virus in certain species of plants. Tobacco and cucumber are almost invariably killed, while the growing points in the pea and the bean are destroyed.

The extreme difficulty we have experienced in infecting healthy lovage plants, either from the original lovage plant or from infected tobacco, is also

of interest. In only one instance out of more than 100 attempts did the lovage plant become infected, yet it is quite easy to obtain 100 per cent infection when the virus is transmitted from lovage to tobacco or from tobacco to tobacco. Owing to this apparent difficulty in transmission one cannot state definitely that some plants, such as celery, which have proved resistant to infection, are actually immune from the virus. There is little doubt, had we obtained the virus in the first place in tobacco instead of in lovage, that we should have considered the latter plant immune and should not have persisted to the extent that we actually did in attempts to infect it.

SUMMARY

A new virus affecting lovage (*Ligusticum scoticum* L.) is described. The host range, so far as tested, seems wide and includes plants in the Solanaceae, Umbelliferae, Leguminosae, Cucurbitaceae, Malvaceae and Cruciferae. The virus is sap-transmissible but its natural means of spread are not known. It is inactivated by a 10-minute exposure to a temperature of 60° C. The infectivity of juice from diseased plants is low.

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"FRECKLE," A SPOTTING OF TOMATO FRUITS[1]

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(Accepted for publication November 19, 1943)

A superficial spotting and blemish of ripe tomato fruits, referred to locally as "freckle," commonly occurs on canning tomatoes in Indiana during the latter part of the season. Affected fruits, although off-color in appearance, are seldom graded lower at the cannery, and as a result the disease causes little loss to the grower.

The spots are typically small, $\frac{1}{8}$ inch or less, and have a dark necrotic center surrounded by a yellow border. The side of the fruits exposed directly to the sun is usually more densely spotted. However, the lower side of some fruits and those in the center of densely foliated plants are often affected. The larger spots or blemishes are dark colored and often lack the chlorotic border. They were particularly common on fruits from plants from segregating generations of *Lycopersicon esculentum* Mill. × *L. pimpinellifolium* (Jusl.) Mill. R. E. Lincoln noted apparent differential varietal response on the tomato fruit in the tomato breeding plot at Lafayette, Indiana. Young lesions of anthracnose (*Colletotrichum phomoides* (Sacc.) Chester) (Fig. 1, C) and certain insect punctures, particularly those made by the stilt bug (*Niedes muticus* (Say)), may be confused with freckle.

Two species of *Alternaria* have been most frequently isolated from the affected areas. The predominance of a particular species varied with the time and location where the freckle fruits were collected. In 1939, *Alternaria solani* (Ell. and Mart.) Jones and Grout was frequently isolated from freckle spots on fruits collected at Lafayette and Battle Ground, Indiana (Fig. 1, D). Since that time this species has only occasionally been found associated with freckle. The organism most frequently recovered was one that Charles Drechsler, who examined the cultures, regards as corresponding generally to the species widely referred to as *Alternaria tenuis* Nees. This fungus has not been pathogenic on tomato foliage. It had been repeatedly isolated from aerial spore traps located at Lafayette, Indiana, in 1938 and 1939.

In 1940 and 1941 occasional attempts were made to reproduce freckle symptoms on ripe fruits in the field and on detached green-mature and ripe fruits in moist chambers in the laboratory by spraying the fruit with a water suspension of mycelium and conidia of the above species of *Alternaria,* but

[1] Cooperative investigations of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Department of Botany and Plant Pathology, Purdue Agricultural Experiment Station. Journal Paper No. 122, Purdue University Agricultural Experiment Station.

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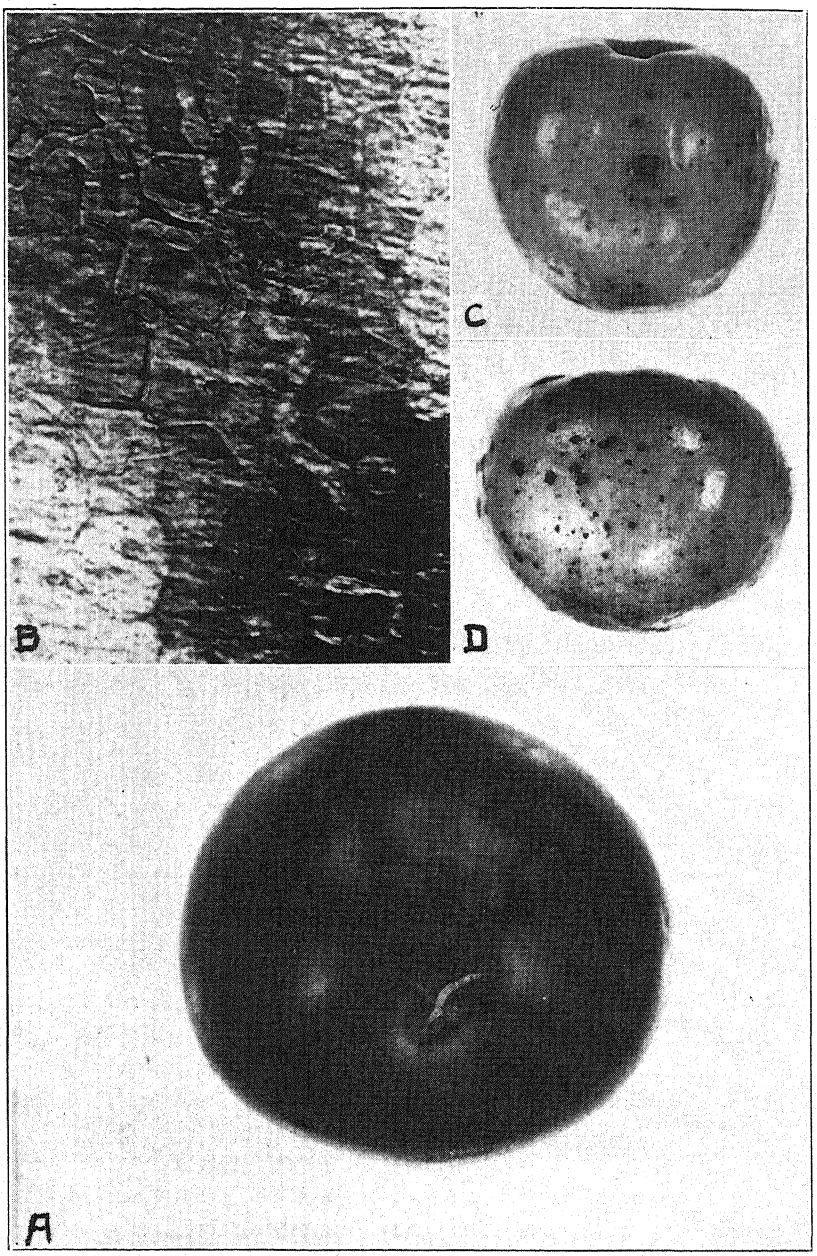


FIG. 1. A. Tomato fruit showing "freckle" symptoms over entire surface. Note chlorotic borders and necrotic centers of spots. B. Fresh mount of epidermal strip from a freckle-affected fruit showing mycelium under the cuticular layer. ×500. C. Tomato fruit showing young anthracnose lesions. D. Young fruit infected by *Alternaria solani* and showing symptoms of a type common in Indiana late in the season.

these attempts were generally unsuccessful. Only a few spots developed and these were all on the green-mature fruit inoculated with *Alternaria solani*. Fruits selected in the field to serve as uninoculated checks frequently developed symptoms during the incubation periods.

In 1942, green, green-mature, pink, and ripe greenhouse fruits were atomized with water suspensions of *Alternaria solani*, *A. tenuis*, a *Penicillium* species, and an unidentified contaminant found in a laboratory culture. The fruits were kept moist for 3 days after inoculation. When *A. tenuis* was used for inoculum, minute spots were visible 4 days after inoculation on the green-mature fruits (then in the pink stage). When the green fruits inoculated with *A. tenuis* began to turn red, spots typical of freckle appeared. Only a few spots developed on the fruits that were ripe at the time of inoculation. The spots on the green fruits resulting from inoculation with *A. solani* were small, raised, and jet black. This type of spotting is typical of infection produced by this fungus on the green fruit in the field, late in the tomato season, and has been described by Gardner.[3,4] Probably only those infections of *A. solani* occurring on the green-mature and ripe fruit are typical of what is referred to as freckle. No spotting was produced by the *Penicillium* species or the unidentified organism.

To study the host-parasite relationships, epidermal strips were removed from freckle-affected fruits obtained in the field. The strips were stained and mounted in a lactophenol solution of acid fuchsin. Mycelium was easily observed in many of the freckle spots (Fig. 1, B). It was in clear focus a short distance beneath the cuticular layer. When paraffin sections were made from epidermal strips and stained with safranin and fast-green, the fungus was observed in a few sections to penetrate through the unbroken cuticle. Many affected areas were located beneath cracks in the skin surface, especially at the bases of broken hairs.

In the affected areas the cells were brown colored and contained brown masses which stained deeply with safranin. What appeared to be oil globules and crystals were observed in the cells. The discoloration seldom extended through more than 3 or 4 layers of cells below the epidermis. Most of the discolored cells, except those in the epidermal layer, were collapsed. The mycelium was observed most commonly in the epidermal layer, but was found also in the cells below. Cell distortion and discoloration apparently occurs in advance of the fungus.

The few attempts to increase the number of infections on the side of the fruit exposed to ultra-violet and infra-red rays were unsuccessful. It is suggested that the predominance of infection on the portion of the fruit exposed to the sun may result from a heavier deposit of air-borne inoculum rather than from any effect from the sun's rays. The greater abundance of freckle late in the season may be explained by the heavier spore load

[3] Gardner, Max W. Indiana plant diseases, 1921. Proc. Ind. Acad. Sci. 33: (1923) 1924.

[4] Gardner, Max W. Indiana plant diseases, 1925. Proc. Ind. Acad. Sci. 36: (1926) 1927.

known to occur in the air at that time, together with the increased exposure of the fruits.

Other fungi may enter the tomato skin and produce a reaction similar to that reported above. Ainsworth *et al.*[5] have described a spotting of tomato fruits caused by a *Botrytis* species under moist conditions. In a study of the structure of tomato skins Groth[6] found patches of brown cells wherever the cuticle or cuticular thickening was cracked, and often the germinating spores of mold fungi were observed in them. The freckle spotting observed in Indiana, however, does not resemble that caused by the organism just mentioned and appears to be due to infection either by *Alternaria solani* or by *A. tenuis*.

[5] Ainsworth, G. C., Enid Oyler, and W. H. Read. Observations on the spotting of tomato fruits by *Botrytis cinerea*. Pers. Ann. Appl. Biol. 25: 308–321. 1937.

[6] Groth, B. H. Alfred. Structure of tomato skins. N. J. Agr. Exp. Stat. Bull. 228. 1910.

MATURE PEACH FRUITS AFFECTED BY LEAF CURL

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(Accepted for publication November 17, 1943)

It appears that *Taphrina deformans* frequently affects the fruits of peach in New York and elsewhere in the United States but is not often observed or recorded on the mature fruits, judging by the paucity of information in the literature. Most records found are of lesions on young or half-mature fruits. In years past the disease has been observed on mature peach fruits at harvest time in New York State by my colleagues Whetzel, Barrus, and Reddick, but not reported. One excellent specimen, preserved in the plant pathology herbarium, was collected in September, 1913. The surface of one fruit was completely involved. Cunningham[1] reported this fungus on mature fruits in New Zealand, adding that this may occur more commonly than is realized.

The first record of lesions on mature peach fruits in the United States appears to be that of Poole[2] who noted affected fruits in the sand hill regions of North Carolina in 1932. He records that when the largest fruits were one-half to three-fourths of an inch in diameter, small hypertrophied leaf-curl lesions were observed confined to the fruit of the varieties Elberta and J. H. Hale, usually on one side and near the calyx end. The disease lesions enlarged throughout the summer, reaching half an inch in diameter. No infections were observed on the early varieties, even when growing in proximity to affected Elberta and J. H. Hale. It is not clear where the inoculum originated nor why the foliage escaped infection. He described the lesions as reddish to purplish in color. Because of no further growth in the diseased areas the fruits were decidedly malformed later in the season because starting in as hypertrophic areas the affected tissues became decidedly atrophic and the surface smooth. Poole[3] mentioned that similar fruit symptoms appeared in Georgia in 1928 and in South Carolina in 1931. The greatest amount of infection encountered was 15 per cent; but, ordinarily, it was less than 2 per cent. Rose, *et al.*,[4] show color reproductions of symptoms on mature fruits. Their suggestion that such symptoms are the result of fungus attacks on maturing fruits seems doubtful.

The discovery that peach-leaf-curl symptoms were exhibited by maturing Elberta fruits in New York in 1943 was made more or less by accident. Due to the previous excessively cold winter very few peaches were produced out-

[1] Cunningham, G. H. Leaf curl, bladder-plum, and cherry-curl. Their appearance, cause, and control. New Zealand Jour. Agr. 26: 85–97. 1923.

[2] Poole, R. F. Late infection of peach leaf curl in the Carolinas. Pl. Dis. Rptr. 16: 171–172. 1932.

[3] See footnote 2.

[4] Rose, D. H., D. F. Fisher, C. Brooks, and C. O. Bratley. Market diseases of fruits and vegetables: peaches, plums, and cherries and other stone fruits. U. S. Dept. Agr. Misc. Pub. 228, 26 pp. 1937.

side of a small area of Niagara County. The peach crop in this area was kept under close observation by the writer during the summer. Although all growers normally spray for leaf-curl control, one grower's Elberta orchard suffered from this disease, which was traceable to the deteriorated sulphur fungicide he used. When examining this orchard early in September it was noted that occasional leaves with curl symptoms minus discoloration still persisted. On closer examination some of the fruits were found to have large reddish lesions covering from one-eighth to three-fourths of the surface area, whereas Poole[3] states that the lesions reached only one-half inch in diameter. The lesions illustrated in figure 1 were relatively shallow, being limited to the outer layers of cells. Many of the lesions developed cracks running usually in a longitudinal or a diagonal direction. The lesions were variable in position, involving areas near the ends of the fruits, strips from end to end, one entire side or one-half the fruit, all one side and

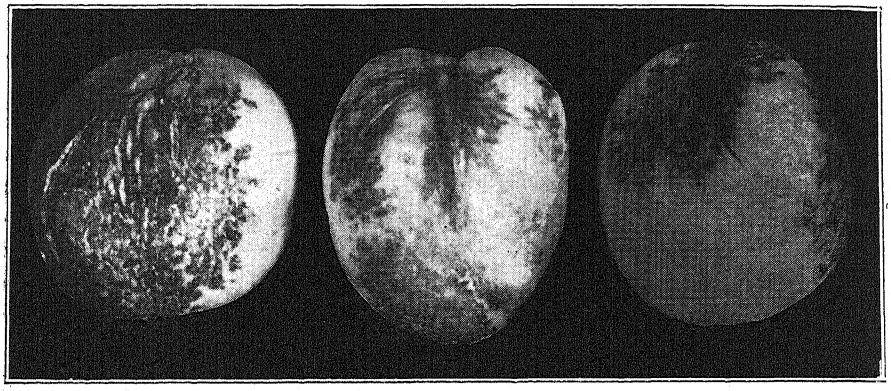


FIG. 1. Leaf-curl symptoms on maturing Elberta peach fruits characterized by striking reddish lesions accompanied by atrophy. Reduced.

portions of the other side, etc. The percentage of fruit affected from tree to tree was ordinarily less than 5 per cent. However, due to prices of 6 dollars or over per bushel, an economic loss resulted in this orchard.

Attempts to demonstrate the leaf-curl fungus in the lesions were all unsuccessful. However, Cunningham,[5] in discussing leaf curl on nectarine fruits in New Zealand states, "Fructifications may develop on these areas, appearing as a delicate bloom; they are unusual, however."

A histological study employing free-hand sections revealed a large reduction in the hairs normally on the surface, which no doubt accounts for the shiny appearance of the affected areas. Cunningham[5] refers to the fact that the lesions ". . . on peaches often appear as if polished, owing to the absence of those hairs which normally cover the surfaces." The reddish, netted discoloration of the lesions on fruits nearing maturity was found to be limited to a shallow layer of cells at the surface, ranging to a depth of about 8 cells. It was further noted that the cells in the region of the lesion

[5] See footnote 1.

were much smaller than normal, possessing thicker walls and more evidence of opaque material within them. Stomates were abundant on both healthy and diseased areas.

In conclusion, the strikingly colorful nature of the lesions observed on mature fruits in New York agree in general with the descriptions of those observed elsewhere. Judging by the nature of its host-parasite relationship, failure to isolate the leaf-curl fungus from old fruit lesions or to observe mycelial elements in connection with them was to be expected. It is not yet clear, however, what the conditions are surrounding the origin and developmental history of lesions on mature peach fruits.

RELATION OF RUST DAMAGE IN SEED FLAX TO SEED SIZE, OIL CONTENT, AND IODINE VALUE OF OIL[1]

H. H. Flor[2]

(Accepted for publication October 22, 1943)

It is estimated that flax rust, *Melampsora lini* (Per.) Lév. reduced the yield of flaxseed in North Dakota in 1942 approximately 25 per cent, or about 2,000,000 bushels. Bison flax, the principal variety grown, is susceptible to North American races of the flax-rust fungus. This variety came into general cultivation in the early 1930's, a period characterized by hot dry seasons, unfavorable for the development of flax rust. Since 1939 rust has become increasingly destructive, and Bison is being replaced by rust-resistant varieties.

Flax rust was generally distributed over North Dakota in 1942, but varied greatly in intensity in different sections of the State and even in different parts of a field. Infection was general in some fields, but in others it was highly variable. Such a variation in intensity occurred in a series of seed-treatment plots at Fargo and offered an opportunity to determine the effect of variations in rust damage on the quantity and quality of oil obtained from the seed.

Bison seed of high quality was used in this test, and the seed treatments with different fungicides had no significant effect on stands. The number of plants ranged from an average of 44 to 55 per foot of drill row. Data secured by Dillman and Brinsmade[3] suggest that in nursery rows, where weeds are not a factor, basal branching compensates for wide variations in stand. In the present tests, actual yields obtained from the various plots corresponded so closely to estimates of the relative injury due to rust, that yields have been used as the criterion of rust damage. Rust-resistant varieties in the nursery yielded about 25 bushels per acre. Rust was estimated to have reduced the yield of the least injured plot of Bison approximately 30 per cent. The yield of the 20 plots ranged from 3.6 to 16.8 bushels per acre. The seed from the different plots ranged in weight from 5.1 to 6.2 grams per 1,000 seeds; in oil content from 36.9 to 39.5 per cent; and in iodine number from 178 to 183.[4] Rust infection was more or less uniform throughout the entire row in the least and in the most severely injured plots. In the plots of intermediate yield, some of the rows showed considerable differences in rust damage; consequently, the harvested seed was a composite from plants suffering severe to moderate rust injury.

[1] Cooperative investigations between Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases.

[3] Dillman, A. C., and J. C. Brinsmade, Jr. Effect of spacing on the development of the flax plant. Jour. Amer. Soc. Agron. 30: 267–278. 1930.

[4] Oil content computed on basis of 8 per cent moisture in the seed. Iodine number determined by refractive index. Analysis by E. P. Painter, Department of Agricultural Chemistry, North Dakota Agricultural Experiment Station.

The simple correlation coefficients between yield, weight of seed, oil content, iodine number, and stand are given in table 1.

High positive correlation coefficients were obtained between yield, weight of seed, and oil content, and high negative coefficients between each of these characters and iodine number of the oil. Correlation coefficients between stand and the other characters, namely, yield, weight of seed, oil content, and iodine number, were within the 1 per cent point and, with one exception, within the 5 per cent point, indicating a lack of significance.

A previous report[5] indicated that a light rust infestation, which reduced yields 25 per cent or less, had little effect on the oil content or quality. In the present tests, no rust-free Bison was available for comparative tests. However, the large seeds (6.2 grams per 1000) and high oil content (39.5 per cent) of the plot yielding at the rate of 16.8 bushels per acre were

TABLE 1.—*Correlation between yield as influenced by rust infection, and weight of 1,000 seeds, oil content, iodine number, and stand of Bison flax at Fargo, N. Dak., 1942*

Characters correlated	Correlation coefficient[a] with			
	Yield	Weight of 1,000 seeds	Oil content	Iodine number
Weight of 1,000 seeds	+0.97	.....	.....	.....
Oil content	+0.96	+0.94	.....	.....
Iodine number	−0.89	−0.83	−0.80	.....
Stand	+0.40	+0.40	+0.49	−0.31

[a] Coefficients above 0.56 are highly significant (beyond the 1 per cent point).

greater than usually obtained from Bison in non-rust years, indicating that moderate rust damage may not adversely affect seed size and oil content.

Dillman and Hopper[6] obtained samples of four varieties grown under many different environments where yield was largely determined by moisture and temperature. They found that, in general, those environmental conditions which reduced yield also reduced seed size, oil content, and iodine number.

In the present study, where differences in yield were due principally to rust infection, although the range in iodine number was small (178 to 183), significant negative correlations were obtained between iodine number and yield, seed size, and oil content. It would appear from these results that when yield is reduced by rust, the iodine number of the oil is not reduced as it is when the flax crop is adversely affected by drought or high temperatures.

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[5] Flor, H. H. Flax rust. N. Dak. Agr. Exp. Stat. Bimo. Bull. 3: 7–9. 1941.

[6] Dillman, A. C., and Hopper, T. H. Effect of climate on the yield and oil content of flaxseed and on the iodine number of linseed oil. U. S. Dept. Agr. Tech. Bull. No. 844. 1943.

BACTERIAL SOFT ROT OF SANSEVIERIA

J. G. Brown and Alice M. Boyle

(Accepted for publication October 10, 1943)

Sansevieria is an ornamental commonly cultivated in greenhouses and also planted outdoors in the warmest parts of the United States. Certain species of the genus, *S. guineensis* and *S. trifasciata* in particular, are grown for their fiber, bowstring hemp, in parts of the tropics. Use of the fiber of *Sansevieria* in a mixture of pineapple and other threads in the manufacture of pina and jusi cloths in the Philippine Islands is remembered by the senior writer. Ornamental planting of *Sansevieria* and commercial utilization of its fiber indicate considerable cultivation of the herb. Nevertheless, we find few references to its diseases and no mention of bacterial maladies affecting any plants of the more than forty species of the genus.

Plants of *Sansevieria trifasciata* growing in the horticultural greenhouse of the University of Arizona were all killed by disease in the winter of 1941–42, although they had been cared for in the usual manner. The leaves became yellowish-green to pale-yellow, developed soft, water-soaked spots at and above the soil line, toppled over sidewise (Fig. 1, *A*), and rotted off at the base (Fig. 1, *C, b, c*). The rootstocks became water-soaked, soft and straw-colored (Fig. 1, *C, d, e*), and the roots died and dried.

From the lesions on leaf and rootstock of several affected plants, colonies of a grayish-white bacterium were repeatedly obtained in pure culture. This bacterium, inoculated into healthy plants of *Sansevieria trifasciata* by the needle-stab method, and also sprayed on the same species, caused symptoms (Fig. 1, *B*) identical with the natural infection. The plants showed evidence of a "take" in 16 days and advanced symptoms of the disease, including many dead plants, in 43 days.

The cause of the soft rot of *Sansevieria* is a grayish-white, actively motile (by 1 and 2 polar flagella; 12-hr. culture, Casares-Gil), Gram-negative, nonspore-forming, slowly gelatine-liquifying, nitrate-reducing, milk-curdling (but nonpeptonizing) short rod, occurring singly or in chains of 2 to 6. Colonies on the surface of potato-dextrose agar plates are circular, elevated, smooth, moist-glistening, with well-defined margin. Growth in nutrient broth is abundant with formation of a pellicle and abundant flaky sediment. Acid curd appears in litmus milk, acid and gas in dextrose broth, and acid in l-arabinose, l-xylose, and raffinose; the reaction is alkaline in cellobiose, maltose, d-sorbitol, levulose, salicin, saccharose, glycerine, lactose, and mannitol. The carbon-source media, respectively, were made by adding 2 per cent by weight of the designated compound, before sterilization, to nutrient broth containing 3 g. of beef extract and 5 g. of peptone per liter.

The bacterium causing rot of *Sansevieria trifasciata, Erwinia carotovora, E. aroideae,* and *E. phytophthora,* which are all soft-rot organisms, are alike in being Gram-negative, aerobes, gelatine liquifiers, nitrate reducers, milk and litmus milk curdlers with acid reactions and no peptonization in the latter, white or grayish white color; acid producers in dextrose, l-arabinose, l-xylose, and raffinose; *E. carotovora* and *E. phytophthora* agree in

failing to produce NH_3 (reaction of *E. aroideae* in this medium appears to be unreported). The *Sansevieria* bacterium is further like *E. carotovora* in producing gas in dextrose, and both grow well on raw carrot; it agrees with *E. aroideae* in producing no indol; no gas in lactose, sucrose, l-xylose, raffinose, maltose, and levulose. Further similarity to *E. phytophthora* is the production of gas in dextrose.

The bacterium of *Sansevieria* soft rot is unlike the other pathogens mentioned above in motility, failure to reduce litmus, acid reaction in lactose and sucrose, and no acid in maltose, levulose, and mannitol; it is unlike both *Erwinia carotovora* and *E. phytophthora* and like *E. aroideae* in its lack of gas formation in lactose, sucrose, l-arabinose, l-xylose, raffinose, and levulose; it is unlike *carotovora* in causing no odor, no gas in lactose, sucrose, l-arabinose, l-xylose, raffinose, levulose, salicin, in growing poorly on steamed sweet potato; unlike *E. aroideae* in producing gas in lactose; further unlike *E. phytophthora* in producing no fringed colonies, and it does not brown or blacken raw potato.

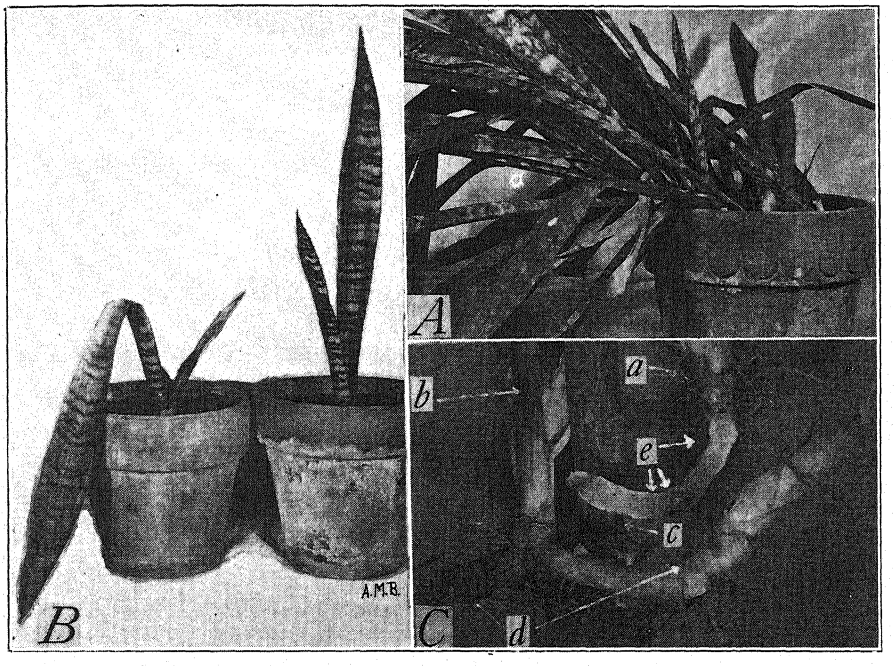


FIG. 1. *A*. Greenhouse plants of *Sansevieria trifasciata* Prain affected by bacterial soft rot. *B*. Inoculated plant, with drooping larger leaf and discolored drying smaller leaf (left). Control injected with sterile distilled water (right). *C*. Parts of naturally infected plants. *a*. Exterior of infected, water-soaked leaf base. *b*. Water-soaked, shrunken part of leaf with base of dead, dried and browned leaf at right. *c*. Lower part of leaf entirely rotted from rootstock. *d*. Exterior of water-soaked, discolored rootstocks to which dead roots and stubs of decayed roots are attached. *e*. Interior, soft-rotted tissues of rootstock.

The bacterium causing the rot of *Sansevieria trifasciata* herein described appears to belong to the group of intermediates mentioned by Stanley,[1] who reports that 19 cultures or 44.2 per cent of 43 cultures isolated from soft rots similarly are intermediate between *Erwinia carotovora* and *E. aroideae* assemblages.

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[1] Stanley, A. R. Physiologic and serologic studies of the soft-rot and colon group of bacteria. W. Va. Agr. Exp. Stat. Bull. 287. 1938.

PHYTOPATHOLOGICAL NOTES

The Pathogenicity of a Nonsporulating Basidiomycete on Grasses in Minnesota[1]—*Fusarium* sp., *Alternaria* sp., and a nonsporulating basidiomycete were isolated from several plants of crested wheatgrass (*Agropyron cristatum*), which had been severely injured by root rot at Clear Lake, Minnesota.[2] Earlier studies[3] had shown *Helminthosporium sativum* P. K. and B. and *Pythium graminicolum* Subr. to be important in causing root rot of this grass in Minnesota. But a nonsporulating basidiomycete had hitherto not been isolated. Pathogenicity of the basidiomycete was tested in the greenhouse by planting seeds of *Agropyron cristatum* (L.) Beauv., *Bromus inermis* Leyss., and *Festuca elatior* L. in steamed soil inoculated with cultures of the fungus grown on a corn meal-soil medium and in noninoculated steamed soil. The tests were made in greenhouses maintained

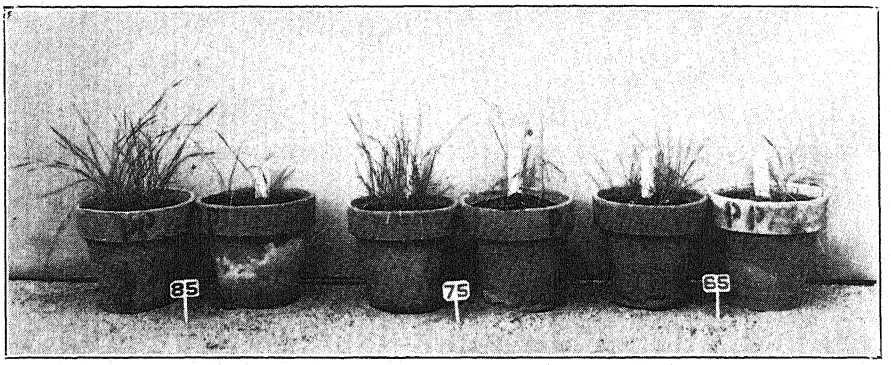


FIG. 1. *Agropyron cristatum* grown in the greenhouse at 85, 75, and 65 degrees F. in steamed soil inoculated with a nonsporulating basidiomycete and in noninoculated soil. The grass growing in inoculated soil is seen on the right at each temperature.

at about 65, 75, and 85 degrees F., respectively. At all these temperatures many seedlings of *Agropyron cristatum* failed to emerge from the inoculated soil and many seedlings were killed after emergence. The most severe injury occurred at 85° F., where only 16 per cent of the plants survived after 22 days (Fig. 1). *Bromus inermis* and *Festuca elatior* were not appreciably affected by the fungus (Table 1). The emergence and survival of these grasses on noninoculated, steamed soil were given a rating of 100 per cent for the purpose of comparison.

The fungus produces a dense white mycelial mat on potato-dextrose agar; the hyphae are from 3 to 6 μ in diameter and bear prominent clamp connections. Neither sclerotia nor spores have yet been observed, although the fungus was grown on several agar media, in water cultures, and in soil.

[1] Published as Paper 2107 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The material was submitted by Henry A. Johnson of the Soil Conservation Service.

[3] Andrews, Edward A. Seedling blight and root rot of grasses in Minnesota. Phytopath. 33: 234–239. 1943.

The organism grows faster and differs culturally from the low-temperature basidiomycete reported by Broadfoot and Cormack.[4] It also is readily distinguishable from cultures of *Typhula itoana* Imai,[5] and does not resemble other *Typhula* spp. described by Remsberg.[6]

The nonsporulating basidiomycete apparently is restricted to the crown of the plant. No lesions were seen on the roots of grasses attacked by this

TABLE 1.—*Susceptibility of Agropyron cristatum, Bromus inermis, and Festuca elatior to a nonsporulating basidiomycete at three temperatures in the greenhouse*

Grass	Emergence, and number of plants surviving 22 days after planting expressed as a percentage of the check[a]					
	65° F.		75° F.		85° F.	
	Emergence	Healthy plants	Emergence	Healthy plants	Emergence	Healthy plants
Agropyron cristatum	69	42	74	42	50	16
Bromus inermis	85	82	92	84	87	71
Festuca elatior	87	86	85	85	103	101

[a] One hundred and fifty seeds of each grass were planted at each temperature.

fungus nor was mycelium observed in any root. In reisolations pure cultures of the fungus were obtained consistently on potato-dextrose agar from the crown of infected plants but not from any other part of the root system.—EDWARD A. ANDREWS, University Farm, St. Paul, Minn.

The Production of Healthy Shoots by Wilted Tomato Plants.[1]—During the past few years the writer has had occasion to observe the behavior of hundreds of tomato plants, inoculated with *Fusarium bulbigenum* var. *lycopersici* and grown in the greenhouse under optimum conditions. Susceptible varieties, such as Bonny Best, usually wilted and died within 3 weeks after inoculation, but occasional plants, though wilting, produced new shoots with no symptoms of disease. These new shoots arose from meristematic regions near the base of the stem and continued to grow even after the main stem had died; they apparently were entirely normal and compared favorably with branches of healthy plants (Fig. 1). Such healthy branches from wilted plants have been observed for 7 weeks after their initial appearance; they produced flowers and even set fruit. The pathogen could invariably be reisolated from the roots and from the wilted main stem of the infected plant, both above and below the origin of the young branch. Only rarely was it isolated from the new shoot, and then only from tissues immediately adjacent to the old stem. Histologic studies showed that the fungus

[4] Broadfoot, W. C., and M. W. Cormack. A low-temperature basidiomycete causing early spring killing of grasses and legumes in Alberta. Phytopath. 31: 1058. 1941.

[5] Cultures of *Typhula itoana* and the low-temperature basidiomycete were obtained from Ian W. Tervet, University of Minnesota.

[6] Remsburg, Ruth E. Studies in the genus *Typhula*. Mycologia 32: 52–96. 1940.

[1] Published as Paper 2108 of the Journal Series of the Minnesota Agricultural Experiment Station.

was present in the vessels of all parts of the main stem, but was exceedingly difficult to find in the new shoot. Occasionally, however, a strand of mycelium could be seen in sections from the base of the branch.

The behavior of these plants is puzzling, for the young branch arises from meristematic tissues of the susceptible parent and its vessels are continuous with those of the crown and roots of the infected plant. The assumption is that the fungus would be free to grow into the vessels of the new shoot or at any rate the toxin produced by the fungus in the lower portions of the old

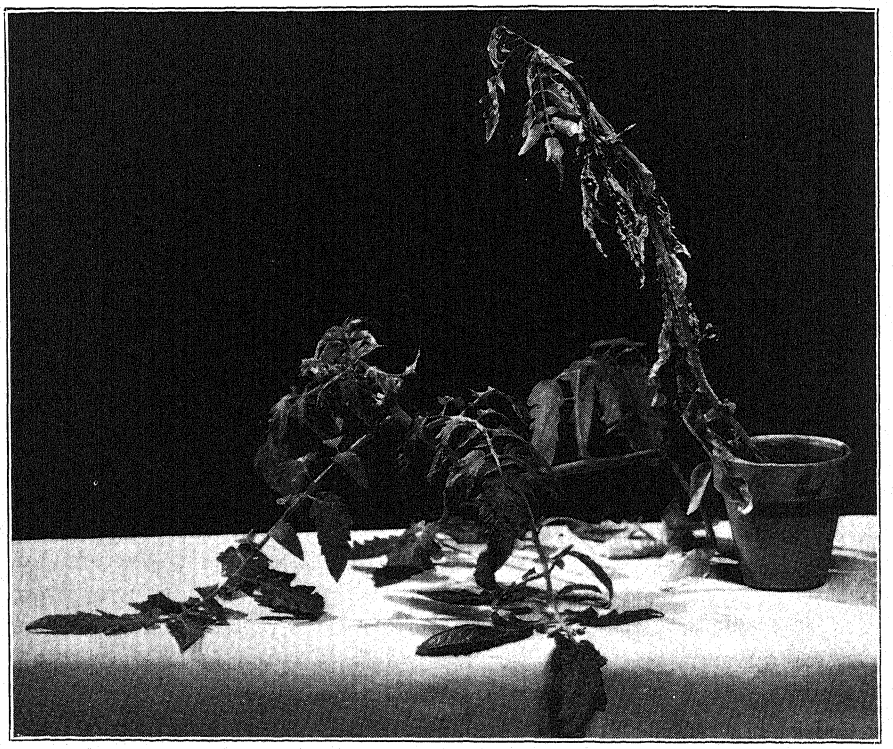


FIG. 1. An inoculated Bonny Best tomato plant showing wilted main stem and vigorous new shoot.

crown could be carried into the new shoot and cause it to wilt. The new shoot, however, appears resistant to the pathogen.—DAVID GOTTLIEB, Agricultural Experiment Station, University Farm, St. Paul, Minnesota.

Low-lime Bordeaux Mixture Controls Leaf Gall on Azaleas.—Azalea leaf gall (*Exobasidium vaccinii* (Fckl.) Wor.) is widespread in this country and Europe, attacking azaleas, rhododendrons, and species of *Vaccinium*. The galls appear on these hosts in the wild, as well as in the nursery and the garden. They have not assumed sufficient importance on cranberries or blueberries to warrant control experiments. On azalea, the galls are commonly so scattered as to excite curiosity only, but in wet seasons or in shaded gardens they may be so numerous as to cause some alarm. Previously pub-

lished accounts of the disease recommend for control the picking and burning of the galls and spraying with 8–8–100 Bordeaux mixture during the period of vegetative growth. The above strength of Bordeaux mixture has been recommended also for control of azalea leaf scorch (*Septoria azaleae* Vogl.), characterized by leaf spots and premature leaf drop.

In one of the writer's pecan experimental blocks near Albany, Georgia, 9 azaleas (*Rhododendron obtusum* (Planch.) Wils.) are planted around 2 of the trees. These pecan trees are the Schley variety, one that is very susceptible to pecan scab, so that 4 spray applications of Bordeaux mixture were necessary to control the disease. During the past 9 years these trees have been sprayed annually with low-lime-Bordeaux mixture as follows:

April 10 to 23—4–1–100 Bordeaux mixture
May 5 to 15—6–2–100 Bordeaux mixture
June 1 to 15—6–2–100 Bordeaux mixture
July 1 to 15—6–2–100 Bordeaux mixture

There was a light infection of the Exobasidium galls on the azalea plants when the spraying was first begun, but the galls soon disappeared and the plants have remained apparently free of all fungus diseases.

Adjacent to the 9 plants mentioned are 290 other azaleas, *Rhododendron obtusum* (Planch.) Wils., *R. macranthum* (Sweet), and *R. mucronulatum* (Blume), all of which carried the Exobasidium disease. A former owner preferred to pick the galls by hand, a practice recommended by local nurserymen; but this was a monotonous job and never really controlled the disease. Recently, however, a new owner took possession of this place and on May 10, 1943, sprayed the 290 diseased plants with 6–2–100 Bordeaux mixture. Fourteen days later most of the galls had become dry and detached from the shrubs. By June 1 the foliage had regained its green color, and some new growth was evident.

During the past 9 years the 9 azaleas have received 36 applications of Bordeaux mixture. There has been no evidence of injury either to flowers or plants. The plants were usually in full bloom when the 4–1–100 Bordeaux mixture was applied. It is possible that the disease could have been controlled by the single application of 4–1–100 Bordeaux mixture in April.

Accumulation of lime with an adverse effect on the pH of certain light soils of the South, might result from high-lime-Bordeaux mixture, such as the 8–8–100 formula. It has been observed also that this type of Bordeaux mixture, at least under certain conditions, may burn the flowers of Indica azaleas, although the leaves are not injured. If Bordeaux mixture is to be applied to azaleas, the 6–2–100 formula seems advisable.

The low-lime-Bordeaux mixture must be carefully prepared, as the margin of safety from copper injury is lower than that afforded by high-lime formulae.—JOHN R. COLE, Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

The Nature of Resistance of Flax to Fusarium lini.[1]—The nature of resistance of Bison C.I. 389 and Punjab C.I. 20 flax to the wilt fungus, *Fusarium lini* Bolley, was investigated by plating on nutrient medium numerous segments of the host taken at intervals after sowing in infested soil in the greenhouse. Cultures of 2 physiologic races of *F. lini* were added to sterilized soil before the seeds were planted. The seedlings were collected daily or every other day, cut into segments ½ to 2 cm. long, depending on size of plant, and plated on nutrient agar.

Bison, grown in soil inoculated with *Fusarium lini* race 6, to which Bison is susceptible, yielded *F. lini* from the primary roots the day the plants emerged, but the fungus was not isolated from the apex of the seedlings until 7 days after emergence. Thereafter, *F. lini* was present in the apex and throughout the entire plant; within 20 days all the plants were wilted. When Bison was grown in soil inoculated with race 11, to which it is resistant, it is significant that *F. lini* was not isolated from the upper parts, although the fungus was universally present in the primary roots near the ground line. In Punjab C.I. 20, which is susceptible to races 6 and 11, the fungus was prevalent throughout the plants a day or two after they emerged.

Thus, the resistant Bison actually does not exclude the pathogen, but confines the less virulent race 11 to the host root and crown tissues and delays the development and spread of the more virulent race 6 for several days. In the susceptible Punjab there is no such limitation or delay, and the fungus develops and spreads rapidly throughout the seedling tissues.

Other data obtained in these experiments indicate that certain varieties of flax may be infected with *Fusarium lini* and not wilt, or wilt only partly; in fact, partial wilting is rather common in certain varieties of flax. Also, plants may be killed to the ground line by *F. lini* and again give rise to healthy shoots. *F. lini* was obtained from segments of wilted branches on plants in which part of the plant remained green and turgid; but the fungus was not obtained from the green parts. Plants of a number of varieties and hybrids that wilted after they had begun to set bolls consistently yielded *F. lini* from the basal portion of the stems to the apex. These results indicate that the fungus must actually be present in the tissue of the stem or branches to induce wilting.—MAX SCHUSTER, Div. of Plant Pathology and Botany, University Farm, St. Paul, Minn.

[1] Published as Paper 2111 of the Journal Series of the Minnesota Agricultural Experiment Station.

ANDRÉS R. LÓPEZ ELÍAS
1909–1943

L. A. Alvarez Garcia

Andrés R. López, for seven years Assistant Plant Pathologist of the Agricultural Experiment Station of the University of Puerto Rico and a member of the American Phytopathological Society, died on February 4, 1943, after an illness of one and a half years.

Mr. López, first son of Mr. Ramón López Linares and Mrs. Teresa Elías de Linares, was born on February 4, 1909, on a coffee farm near San Sebastián, Puerto Rico, and attended elementary school in that town. He entered the High School Course of the Polytechnic Institute, at San Germán, P. R., where he was graduated in the year 1928. In the same year he entered the College of Agriculture and Mechanic Arts of the University of Puerto Rico, where he began the general course in agriculture. At the end of his first year he enrolled at Louisiana State University, where he received the degree of Bachelor of Science in Agriculture in 1933. Continuing his post-graduate studies, he was awarded in 1935 his M.S. degree, with a major in Plant Pathology. While at Louisiana he had opportunity to acquaint himself with the different types of sugar-cane mosaics under study at the University Experiment Station. This experience later proved of value in his work and in the preparation of his unpublished thesis on mosaic resistance of different cane varieties.

Upon his return to Puerto Rico he started work as Agronomist in the Federal Emergency Relief Administration, and, later, taught vocational agriculture at one of the Second Unit Schools of the Department of Education at San Sebastián, Puerto Rico. He joined the technical staff of the Experiment Station of the University of Puerto Rico in 1936 as Assistant Plant Pathologist, thus coming to work in the particular line of studies that had been his chosen vocation. Greatly interested in his work, he attended Iowa State University during 1939–40, where he pursued further phytopathological studies.

Shortly after resuming his duties at the Insular Experiment Station he married Miss Belén H. Cestero, from Vega Baja, Puerto Rico, and but a few months later was taken seriously ill from a childhood infection of Bilharzia from which he never recovered.

With his death both the agricultural experiment station and the agriculture of the Island lose a genuine scientist of great promise, and his colleagues a true and sincere friend.

STATISTICAL STUDIES OF DISTRIBUTION OF PSOROSIS-AFFECTED TREES IN CITRUS ORCHARDS[1]

A. A. BITANCOURT[2] AND H. S. FAWCETT[3, 4]

(Accepted for publication December 15, 1943)

INTRODUCTION

In a number of citrus orchards in California, careful records of psorosis-affected trees have been kept by orchard managers and tree surgeons. These records, as represented on maps on which the diseased trees have been charted, furnish excellent material for statistical analysis. This analysis has thrown light on the distribution and probable manner of spread of this disease in orchards.

As shown by Fawcett (3, 4), psorosis of citrus is a virosis that manifests itself by leaf symptoms of the mosaic type and by bark lesions. On young leaves both the veinlets and the adjacent tissue show faint to pronounced clearing. These small cleared areas, about 1 to 2 mm. long and ½ to 1 mm. broad, may be numerous and scattered over the entire leaf blade, or they may be limited to certain portions. Frequently, a distinct pattern, such as the well-known "oak-leaf pattern," is formed. These cleared areas usually disappear as the leaf matures. Bark symptoms rarely occur on trees less than 6 to 8 years of age. They usually begin either as scales of bark or as aggregations of small pustules, under which are brown specks, on the surface of the older bark of trunks or limbs. As the scaling advances, the deeper layers of bark, and eventually the wood, become affected. A considerable amount of gum occurs near the cambium and between layers of wood, some of which is pushed to the surface (7). The wood in later stages becomes light drab to brown or reddish brown, the stain progressing in an irregular fashion, not necessarily following the grain of the wood (2). Although the leaf symptoms have considerable importance in connection with the study of the disease and are of practical importance for the identification of diseased trees, the bark symptoms are the only ones that are readily recognized. The records used for the present studies were, accordingly, those of diseased trees already showing bark symptoms.

[1] Paper No. 501, University of California Citrus Experiment Station, Riverside, California.

Part of this paper was presented at the twenty-sixth annual meeting of the Pacific Division of The American Phytopathological Society, Salt Lake City, Utah, June 17 to 19, 1942 (1). The work reported in this paper was begun during the tenure, by the senior writer, of a John Simon Guggenheim Memorial Foundation Fellowship for research at the University of California Citrus Experiment Station, Riverside, in 1942, and was completed in 1943.

[2] Plant Pathologist, Instituto Biologico, São Paulo, Brazil.

[3] Professor of Plant Pathology and Plant Pathologist in the Experiment Station, University of California.

[4] The writers wish to express their appreciation to Messrs. P. S. Sloop, O. W. Murray, Homer Barnes, J. C. Perry, and T. A. Lombard for supplying maps and careful records of the distribution of psorosis-affected trees in the orchards under their management; and to Dr. E. R. Parker and Dr. F. M. Turrell for their criticism of the manuscript.

Observational and experimental evidence have shown that the transmission of psorosis is mostly due to the use of buds from diseased parents. Based on this knowledge, a system of registering healthy parent trees has been established in California. Trees thought to be healthy are tested according to certain rules involving the observation of both leaf and bark symptoms, and, if the test indicates that they are healthy, they are registered by the State Department of Agriculture. The propagator is then given evidence of registration, which may be shown to the buyers of trees budded from the registered parents (5, 6).

In the past few years, however, there has been strong indication that bud transmission alone could not account for all the psorosis-affected trees ob-

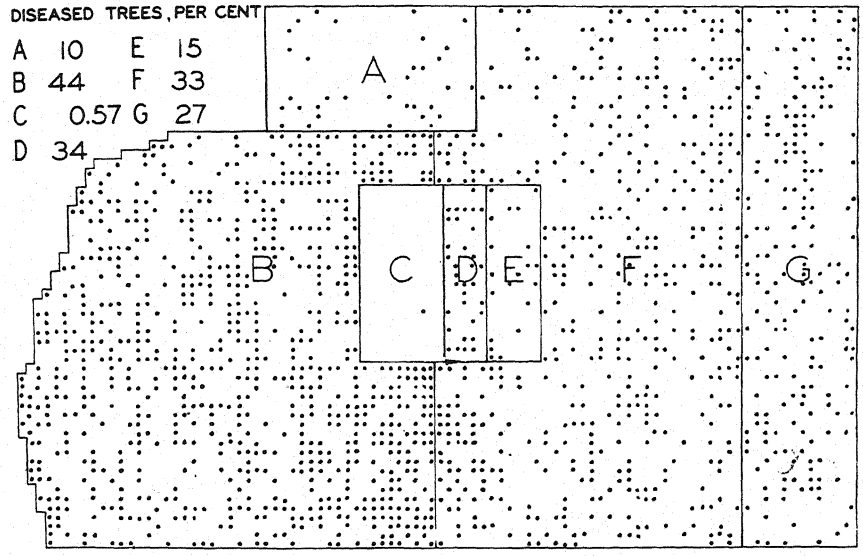


FIG. 1. Types of stencils used in counting diseased trees around diseased trees and diseased trees around healthy trees at distances L (A), 1.4 L (B), 2 L (C), and 2.8 L (D).

served in some orchards. For example, in a 35-year-old Valencia orchard in Orange County, 9 out of 10 new cases reported in 1939 developed on supposedly healthy trees that were adjacent to one or more diseased trees. In another orchard in Orange County, a planting of about 500 trees, the buds of which originated from 1 healthy tree, 4 trees have developed bark symptoms. In an orchard in Ventura County, where the origin of each tree that has been planted in the last 18 years has been carefully recorded, a small percentage (about 0.3 per cent) of trees budded from trees still living and that do not show any symptoms of psorosis, have developed the bark symptoms during the past few years. Similar evidence has been found in several other orchards.

In the majority of orchards in California, the trees of each orchard have originated from buds from a number of parent trees. Since in recent years

only has it been known that psorosis is caused by a virus transmitted by budding, a considerable number of younger trees with psorosis virus in them, but showing no bark symptoms, were used unwittingly by nurserymen as bud-parent trees. The percentage of psorosis-affected trees will vary from one planting to another, according to the relative numbers of diseased trees furnishing buds for propagation. When whole sections of a given orchard are composed of trees from different sources, very striking differences are observed, which can only be accounted for when those sources are known. An example is the orchard represented by the map in figure 1, 7 sections of which are known to have come from 7 different sources. The percentages of psorosis-affected trees in these sections were found to be 0.57, 10.0, 15.0, 27.0, 33.0, 34.0, and 44.0, respectively. The statistical method used in this paper cannot be applied to such heterogeneous orchards as a whole.

If the trees of an orchard come from different parent trees, some of which are healthy and some of which have psorosis, the conditions of propagation and planting in the orchard may have been such as to completely randomize the position of the diseased trees. This is probably the case in most orchards. The bud sticks usually were gathered by the nurserymen from several parent trees, with and without the virus in them. The sticks were tied into a bundle, with no attempt to keep separate the sticks from different parents. The sticks were further shuffled when the bundle was untied in the nursery and sticks were picked, one after another, in the process of budding the trees. As a result, whereas there was a certain amount of grouping in the nursery, owing to the fact that usually all the buds from the same stick were budded in a continuous series of trees, the sticks themselves were already more or less randomized when budding was performed. In the operations of digging out the trees, carrying them to the orchard, and planting them, there occurred several new opportunities for further randomization, so that the final position of the diseased trees in the orchard was random.

While such a random distribution of the diseased trees would remain the same in the absence of contamination of healthy trees by diseased trees, with spread of the disease there would be an increase in the number of diseased trees around the originally diseased trees, which would be determined by statistical analysis.

MATERIALS AND METHODS

The present study was based on the distribution of psorosis-affected trees, as recorded on maps of 14 orchards in Ventura, Orange, Riverside, and San Bernardino counties. The trees in these orchards were of miscellaneous parentage, and the percentages of diseased trees ranged from 4.8 to 70.6. Statistical methods were used to disclose possible grouping of trees that could only be accounted for by the transmission of psorosis from the diseased trees to the healthy trees in an orchard.

Most of the maps represented rectangular orchards, or rectangular parts of irregular orchard maps. Orchard 5 (Table 1) was made up of 2 sections,

TABLE 1.—*Mean incidence of psorosis-affected trees at distance L from central trees,*[a] *determined on maps of the various orchards*

Orchard No.	Age of trees, in years	Total number of trees	Percentage of diseased trees[b]	Calculations based on:									
				Four trees			Two trees						
							Along rows			Along arrays			Difference,[c] a and b
				Diseased	Healthy	Difference[c]	Diseased (a)	Healthy	Difference[c]	Diseased (b)	Healthy	Difference[c]	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	15	2159	4.8	0.174	0.189	− 0.015	.....	.....	.....	.....	.....	.....	.....
2	50	1422	7.6	0.307	0.293	0.014	.....	.....	.....	.....	.....	.....	.....
3	16	1419	10.2	0.408	0.402	0.006	.....	.....	.....	.....	.....	.....	.....
4	20	2756	10.3	0.570	0.395	0.175**	0.357	0.190	0.167**	0.213	0.206	0.007	0.144**
5	22	2753	13.8	0.633	0.540	0.093*	0.292	0.273	0.019	0.341	0.264	0.077**	− 0.049
6	33	911	14.9	1.193	0.498	0.695**	0.568	0.252	0.316**	0.633	0.246	0.387**	− 0.065
7	32	1430	18.7	0.848	0.713	0.135*	0.408	0.358	0.050	0.450	0.345	0.105*	− 0.042
8	29	702	32.0	1.484	1.181	0.303**	0.703	0.615	0.088	0.781	0.566	0.215**	− 0.078
9	29	648	35.6	1.648	1.260	0.388**	0.791	0.650	0.141*	0.852	0.613	0.239**	− 0.061
10	29	702	39.6	1.887	1.387	0.500**	0.895	0.738	0.157**	0.992	0.660	0.332**	− 0.097
11	21	946	42.8	1.869	1.586	0.283**	0.952	0.789	0.163**	0.923	0.797	0.126**	0.029
12	29	675	52.5	2.427	1.747	0.680**	1.192	0.883	0.309**	1.235	0.875	0.360**	− 0.043
13	32	570	61.6	2.577	2.251	0.326**	1.297	1.120	0.177**	1.280	1.136	0.144**	0.017
14	29	1029	70.6	2.924	2.554	0.370**	1.504	1.195	0.309**	1.422	1.359	0.063	0.082*

[a] For explanation of "central" trees and procedure used, see "Materials and Methods."
[b] Based on the number of central trees.
[c] * = significant (5 per cent level); ** = highly significant (1 per cent level).

1 rectangular and 1 slightly irregular; orchard 7 was made up of 4 small sections; orchard 6 of 2 sections, 1 rectangular and 1 irregular, of which only the rectangular part was used. Maps 8, 9, 10, and 12 represented 4 almost square blocks of a single orchard. They, however, showed different percentages of diseased trees and were, therefore, treated separately. The number of trees in the orchards ranged from 570, in orchard 13, to 2756, in orchard 4 (Table 1).

In orchards planted in squares (the only type used in the present investigations), there are 8 trees adjacent to every tree (Fig. 3), except those in

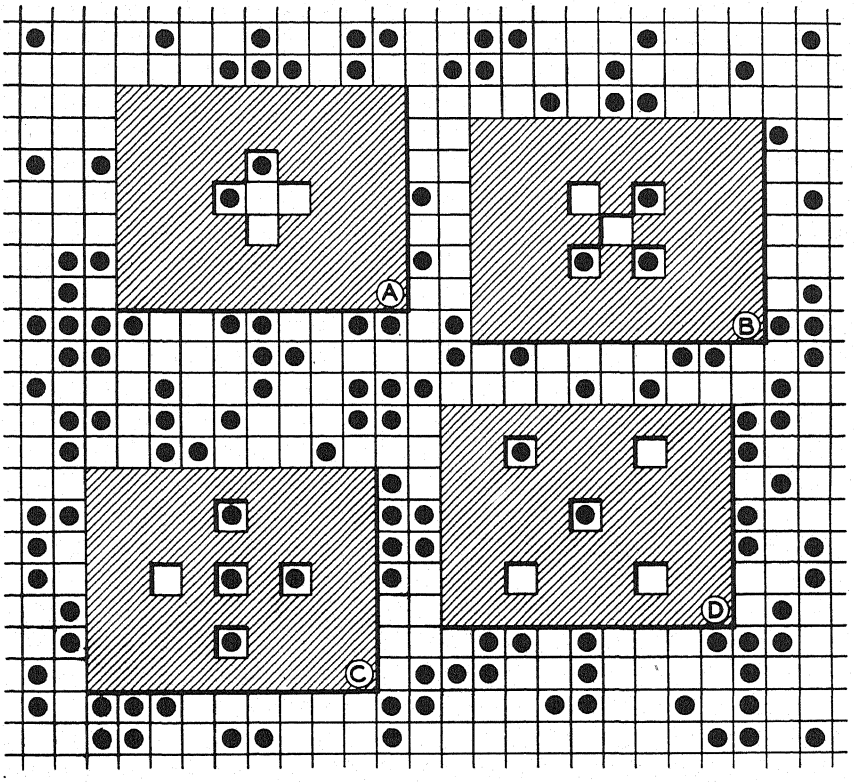


FIG. 2. Map showing distribution of psorosis-affected trees (black dots) in an orchard in Ventura County, California. The irregularities in the percentages of diseased trees in various sections are due to the fact that the buds of these trees came from seven different sources.

the borders. If the distance of each of the 4 trees closest to the central tree is L, then the distance of the 4 other trees is $L\sqrt{2}$, or approximately 1.4 L. Next to these 8 adjacent trees are 16 trees, of which only 8 have been considered in the present study, that is, those at a distance 2 L and 2.8 L from the central tree.

It seems reasonable to assume that if a central tree is diseased and has contaminated some of the adjacent trees, the number of diseased trees around it would be greater than around a central healthy tree.

The counting of diseased trees around diseased trees and of diseased trees around healthy trees was made on the maps with the aid of appropriate stencils (Fig. 2). Each stencil, cut from a piece of paper, disclosed 5 trees, namely, the central tree and 4 others at a given distance (L, 1.4 L, 2 L, or 2.8 L). On the tally sheet, there were, for each of these distances, 2 columns, 1 for diseased trees around diseased central trees and 1 for diseased trees around healthy central trees. In figure 2, A, for example, it may be seen that the central tree is healthy, and that there are 2 healthy trees and 2 diseased trees at distances L from the central tree. Accordingly, in this case, the entry on the tally sheet, under "distance L, healthy central trees," was "2." The stencil was then moved on to the next tree in the row, and the reading for diseased trees was made and entered on the tally sheet, and so on, until readings had been recorded for all the trees available for this stencil. The frequencies were then summed up, and the means of the two columns under "distance L," their difference, and its error were calculated. Similar readings and calculations were made for each of the other distances, 1.4 L, 2 L, and 2.8 L.

RESULTS

For each of the 14 orchards, the mean incidence of psorosis-affected trees adjacent to diseased and to healthy trees, and their mean differences, at the various distances, are shown in tables 1 and 2.[5]

The percentages of diseased trees shown in tables 1 and 2 are based on the number of central trees used in each calculation and are slightly different for different distances. While most of the trees in the orchards, in applying any given stencil, were used once as central trees and 4 times as trees at the given distance from central trees, certain border trees in this calculation were not used as central trees because they did not have 4 trees around them at that distance. The diseased trees in these border rows, not used as central trees for a given distance, were not counted for the total percentage of diseased trees for that distance. Border trees (in any rectangular orchard) were used from 1 to 3 times, both at distance 1.4 L and at distance 2.8 L from central trees, according to position. At distance L, however, there is 1 tree at each corner, and at distance 2 L there are 4 trees at each corner, not used at all. Whereas there are only 2 rows of border trees that are used less than 4 times around central trees at distances L and 1.4 L from central trees, there are 4 rows at distances 2 L and 2.8 L.

The "t" test was used to ascertain the significance of the differences (8). In the tables, highly significant differences corresponding to a probability of 1 per cent, or less, are marked with two asterisks; significant differences, between the 5 and 1 per cent levels, are marked with one asterisk. It may be seen in table 1 that, for orchard 1, the mean number of diseased trees is slightly, but not significantly, larger around healthy trees than around diseased trees. All other orchards show a greater mean number of diseased

[5] All calculations were made with a slide rule, and the means are affected with errors of not more than 0.2 per cent.

TABLE 2.—*Mean incidence of psorosis in trees at distances 1.4 L, 2 L, and 2.8 L from diseased and healthy central trees,*[a] *determined on maps of the various orchards*

Orchard No.	1.4 L				2 L				2.8 L			
	Percentage of diseased trees[b]	Mean incidence			Percentage of diseased trees[b]	Mean incidence			Percentage of diseased trees[b]	Mean incidence		
		Diseased trees	Healthy trees	Difference[c]		Diseased trees	Healthy trees	Difference[c]		Diseased trees	Healthy trees	Difference[c]
4	10.3	0.407	0.415	− 0.008	10.3	0.476	0.416	0.060	10.3	0.480	0.420	0.060
5	13.9	0.623	0.533	0.090*	13.8	0.643	0.525	0.118**	13.7	0.575	0.531	0.044
6	14.9	1.211	0.503	0.708**	15.6	0.922	0.548	0.374**	15.6	0.798	0.550	0.248**
7	18.7	0.758	0.715	0.043	18.8	0.702	0.727	− 0.025	18.8	0.739	0.717	0.022
8	32.0	1.349	1.248	0.101	31.2	1.329	1.244	0.085	31.2	1.266	1.302	− 0.036
9	35.6	1.597	1.249	0.348**	34.6	1.610	1.263	0.347**	34.6	1.283	1.419	− 0.136
10	39.6	1.731	1.508	0.223**	39.6	1.740	1.451	0.289**	39.6	1.620	1.490	0.130
11	42.8	1.906	1.554	0.352**	43.3	1.796	1.668	0.128	43.3	1.734	1.651	0.083
12	52.5	2.285	1.930	0.355**	52.4	2.297	1.926	0.371**	52.4	2.225	2.039	0.186
13	61.6	2.512	2.350	0.162	62.6	2.512	2.322	0.190	62.6	2.418	2.336	0.082
14	70.6	2.877	2.638	0.239**	70.2	2.906	2.539	0.367**	70.2	2.863	2.645	0.218**

[a] For explanation of "central" trees and procedure used, see "Materials and Methods."
[b] Based on the number of central trees.
[c] * = significant (5 per cent level); ** = highly significant (1 per cent level).

trees at distance L from diseased trees than at distance L from healthy trees. Except for orchards 2 and 3, the differences found are all significant or highly significant statistically.

Substantially the same results were obtained at the 3 other distances (Table 2). Orchards 1, 2, and 3 were not treated, but results for the other orchards show that the numbers of diseased trees around diseased trees as centers are usually significantly greater statistically than the numbers of diseased trees around healthy trees as centers. Negative differences were observed, however, at distance 1.4 L for orchard 4, at distance 2 L for orchard 7, and at distance 2.8 L for orchards 8 and 9. Furthermore, differences were statistically significant, or highly significant, in only 7 of the 11 orchards at distance 1.4 L, in only 6 orchards at distance 2 L, and in only 3 at distance 2.8 L. The tables also show that there is usually a marked decrease in the differences as the distance increases. The means of all the differences, and their standard errors, not counting the negative differences, are 0.305 ± 0.022, for 13 orchards at distance L; 0.262 ± 0.025 and 0.233 ± 0.027, for 10 orchards at distances 1.4 L and 2 L, respectively; and 0.119 ± 0.027, for 9 orchards at distance 2.8 L.

The decrease of the differences, per unit distance L (assuming that the difference at central tree is 1), is 0.695 between the central trees and the trees at distance L; 0.108 between trees at distances L and 1.4 L; 0.049 between trees at distances 1.4 L and 2 L; and 0.142 between those at distances 2 L and 2.8 L. As will be seen later, this decrease per unit distance from the central tree may represent a mean gradient of infectivity.

GROUPING OF DISEASED TREES ALONG ROWS OF PLANTING

The method that has just been described therefore discloses a grouping of diseased trees.[6] Such a grouping of trees might be due to the fact that trees originating from the same diseased parents were kept together during the several operations from the budding in the nursery to the planting in the orchard. It is easily seen that grouping due to such a cause would increase the differences in the numbers of diseased trees around diseased trees, and around healthy trees, only for the distances L and 2 L. For the distances 1.4 L and 2.8 L, that is, along the diagonals of the orchard, no such effect would be produced in planting. Furthermore, if the grouping of trees is due to this cause, it would take place along only one of the two general directions of rows of trees in the orchard, that is, along rows or along arrays, according to the direction in which the orchard was planted, and analysis as above, using the 2 trees at distance L from central trees along rows, and the 2 trees at distance L from central trees along arrays, separately, would disclose the fact. This analysis in the 2 directions at right angles to each other was made for all the orchards mentioned in table 1, except orchards 1,

[6] The same grouping also might be shown by fitting a theoretical random distribution, such as the binomial or the Poisson distributions, and applying the χ^2 test of goodness of fit. This method was used extensively in the course of the present study, but, inasmuch as it merely confirms the conclusions reached by the "t" test without giving a measure of the differences between diseased and healthy trees, the results obtained are not given here.

2, and 3, which in the previous analysis had not shown significant differences at distance L. The results have been entered in columns 8 to 13 of table 1. In column 14 the differences between the mean numbers of diseased trees at distance L from diseased trees, along rows and along arrays, have been entered.

It may be seen in table 1 that in orchards 6, 9, 10, 11, 12, and 13, there were significant or highly significant differences between the mean numbers of diseased trees around diseased trees and of diseased trees around healthy trees, both along rows (col. 10) and along arrays (col. 13). The differences between the numbers of diseased trees at distance L from diseased trees along rows and along arrays (col. 14) were not significant. It can be assumed that the diseased trees from the nursery were thoroughly randomized when planted in those orchards. No significant differences were found along rows in orchards 5, 7, and 8, or along arrays in orchards 4 and 14. Only in orchards 4 and 14, however, were there significant differences between the numbers of diseased trees on the 2 sides of diseased trees along rows and along arrays (col. 14). Only in those 2 orchards, therefore, is it safe to assume that there was grouping of diseased trees at the time of planting.

ANALYSES OF FICTITIOUS ORCHARDS COMPARED WITH THOSE OF ACTUAL ORCHARDS

As previously stated, the purpose of the present study was to disclose grouping of diseased trees that could be accounted for only by transmission of psorosis from diseased to healthy trees. In order to ascertain the effect of such transmission (to a known number of trees at different distances from given trees) on the differences calculated as described above, maps of fictitious orchards were prepared. Dots representing psorosis-affected trees, as in figure 2, were distributed on these maps according to the method described below.

Maps I, II, and III represented square orchards, map I with 50 rows of 50 trees each, and maps II and III with 33 rows of 33 trees each. Map IV had the same layout as orchard 11 (Tables 1 and 2), namely, 22 rows of 43 trees each. On each of these maps, a number of dots representing "diseased" trees were distributed by drawing at random, first the number of a row, from one set of numbered cards, and then the number of a tree, from another set. Counts and calculations were then made for distance L on all four maps, and also for the other three distances on map I (Table 3). In the next step, a given number of dots representing "contaminated" trees were placed at random at distance L from the other dots representing "diseased" trees. This was done by drawing at random, first the number of a row, then the number of a "diseased" tree, or dot, and then the direction, north, south, east, or west. Counts and calculations were then again made for distance L on maps I, II, and III. Finally, the same procedure was used for placing given numbers of dots ("contaminated" trees) at the other 3 distances (1.4 L, 2 L, and 2.8 L) from the "diseased" trees, and the same method of calculation was again applied.

TABLE 3.—*Data obtained from maps of fictitious orchards, on which given percentages of "contaminated" trees were placed at random at distances L, 1.4 L, 2 L, and 2.8 L from randomized "diseased" trees and analyzed as in tables 1 and 2*

Map and count Nos.	Distance from central tree	Percentage of contaminated trees	Percentage of diseased trees[a] (p)	Mean incidence			$\frac{pd}{4}$
				Diseased trees	Healthy trees	Difference[b] (d)	
1	2	3	4	5	6	7	8
Map I							
Count 1	L	0	8.03	0.270	0.325	−0.055	
	1.4 L	0	8.03	0.373	0.316	0.057	0.11
	2 L	0	7.99	0.355	0.315	0.040	0.08
	2.8 L	0	7.99	0.367	0.314	0.053	0.11
Total		0					0.30
Count 2	L	5	13.03	1.230	0.414	0.816**	2.66
Count 3	L	5	19.1	1.291	0.635	0.656**	3.13
	1.4 L	3	19.1	1.209	0.652	0.557**	2.64
	2 L	2	18.8	1.033	0.688	0.345**	1.62
	2.8 L	1	18.8	0.940	0.717	0.223**	1.05
Total		11					8.44
Map Ia							
	L	5	19.1	1.443	0.597	0.846**	4.03
	1.4 L	3	19.1	1.223	0.646	0.577**	2.76
	2 L	2	18.8	0.964	0.708	0.256**	1.20
	2.8 L	1	18.8	0.975	0.706	0.269**	1.23
Total		11					9.22
Map II							
Count 1	L	0	15.2	0.603	0.600	0.003	0.0
Count 2	L	9	24.2	1.474	0.793	0.681**	2.6
Count 3	L	9	32.7	1.653	1.122	0.531**	4.3
	1.4 L	5	32.7	1.538	1.161	0.377**	3.1
	2 L	3	32.2	1.522	1.179	0.343**	2.8
	2.8 L	0	32.2	1.296	1.286	0.010	0.1
Total		17					10.3
Map III							
Count 1	L	0	19.5	0.770	0.782	−0.012	.....
Count 2	L	15	34.4	1.866	1.125	0.741**	6.4
Count 3	L	15	54.7	2.409	1.893	0.516**	7.0
	1.4 L	10	54.7	2.278	2.044	0.234**	3.2
	2 L	5	54.8	2.396	1.906	0.490**	6.7
	2.8 L	3	54.8	2.226	2.081	0.145*	2.0
Total		33					18.9
Map IV							
Count 1	L	0.0	42.8	1.692	1.713	−0.021	.....
Count 3	L	13.8	77.3	3.125	2.967	0.158	3.1
	1.4 L	7.8	77.3	3.202	2.712	0.490**	9.5
	2 L	7.8	79.8	3.200	2.923	0.277**	5.5
	2.8 L	3.8	79.8	3.125	2.993	0.132	2.6
Total		33.2					20.7

[a] Based on the number of central trees.
[b] * = significant (5 per cent level); ** = highly significant (1 per cent level).

Map Ia was identical with map I, except that 50 per cent of the trees placed at distance 1.4 L from the diseased central trees were placed entirely at random, and 50 per cent were placed next to contaminated trees at distance L from the central trees, while the contaminated trees at distances 2 L and 2.8 L from central trees were so placed as to be adjacent to contaminated trees at distances L and 1.4 L, respectively, from central trees. This map, therefore, reproduces the condition of an orchard in which the disease would have been transmitted to trees at distances 2 L and 2.8 L from central trees indirectly through already contaminated trees at distances L and 1.4 L, respectively, from diseased central trees. Half the trees at 1.4 L from central trees presumably would have been contaminated directly by the diseased central trees, and half indirectly through contaminated trees at distance L from the central trees.

Results of analyses of maps of fictitious orchards are shown in table 3. It is seen that, as might have been expected, the first randomization (count 1) did not show significant differences between the mean numbers of diseased trees around diseased trees and around healthy trees. When, however, dots representing "contaminated" trees were placed at given distances from "diseased" trees, significant differences appeared.

Comparison of studies of maps I and Ia (Table 3) shows that, when "contamination" of trees at distances 1.4 L, 2 L, and 2.8 L is not the result of direct transmission from diseased central trees, the differences for distance L are increased. The same undoubtedly also applies to distance 1.4 L, but did not show in the case of maps I and Ia, owing to the comparatively large errors affecting these differences.

Comparison of the results from the fictitious orchards (Table 3) with those from the actual orchards (Tables 1 and 2) shows that map I was somewhat comparable to orchard 6, and map III to orchard 12 (Table 4). All the actual orchards showed smaller differences than the fictitious orchards, but not substantially so in several cases. Orchard 6 was the only one in which a very conspicuous grouping could be detected on the map. Grouping in the other orchards was not readily detected by simple examination of the maps.

DISCUSSION

From the results obtained with the fictitious orchards, it must be concluded that, assuming random distribution of diseased trees at the time of planting in an orchard, a later grouping of diseased trees, revealed by the method reported in this paper, indicates a subsequent transmission of disease to healthy trees. The method reveals groupings that cannot be detected readily by looking at a map.

If, instead of using the same trees once as central trees and several times as adjacent trees, each tree had been used only once, that is, if the orchard had been divided into groups of 5 trees, each group consisting of a central tree and the 4 adjacent trees at the given distance, the percentage of contaminated trees could have been calculated. We have seen that, in

TABLE 4.—*Differences and estimated percentages of contaminated trees of actual and fictitious orchards—orchard 6 compared with map I and orchard 12 compared with map III (see Tables 2 and 3)*

Distance	Orchard 6		Map I		Distance	Orchard 12		Map III	
	Difference	Percentage of contaminated trees	Difference	Percentage of contaminated trees		Difference	Percentage of contaminated trees	Difference	Percentage of contaminated trees
L	0.695	2.6	0.656	3.1	L	0.680	8.9	0.516	7.0
1.4 L	0.708	2.6	0.557	2.6	1.4 L	0.355	4.7	0.234	3.2
2 L	0.374	1.5	0.345	1.6	2 L	0.371	4.9	0.490	6.7
2.8 L	0.248	1.0	0.223	1.1	2.8 L	0.186	2.4	0.145	2.0
		—		—			—		—
Total		7.7		8.4	Total		20.9		18.9

the absence of contamination, no significant difference was found between the mean number of diseased trees adjacent to diseased trees and the mean number of diseased trees adjacent to healthy trees. With due allowance for the effect of random errors, the difference, d, found when contaminated trees were added to the map, was, therefore, due to those trees. The probability of contamination of any one of the 4 adjacent trees by the central tree is, therefore, d/4, and if p represents the percentage of diseased central trees, pd/4 represents the percentage of trees contaminated by central trees at the given distance. However, inasmuch as the same trees are used as central trees and as adjacent trees, p and d are correlated, and the product is smaller than the actual percentage of contamination. In table 3 the determinations pd/4 have been entered in the last column and can be compared with the actual percentages of "contaminated" trees distributed on the maps. While there are minor variations owing to the comparatively large errors affecting the results, the formula in most cases gives results comparable to, although smaller than, the actual percentages of contamination.

The results reported for map Ia (Table 3, col. 8), for all distances except 2 L, are greater than those reported for map I. It is easily understood why the results for distance L, for instance, should be greater in map Ia, reproducing conditions of indirect transmission, than in map I, in which a perfect random distribution of contaminated trees at the 4 distances results in independent values for the 4 differences. In the case of map Ia, transmission of the disease from the central tree to a tree at distance 2 L is through the intermediate tree at distance L from both. The difference for distance L will, therefore, contain the effect of the transmission from the central tree to the tree at distance L and to the tree at distance 2 L; for the same reason, it will also contain the effect of transmission to a tree at distance 1.4 L. Similarly, the difference for distance 1.4 L will also contain the effect of transmission to distance 2.8 L. For maps I, II, III, and IV, an estimate of the total percentage of contaminated trees, therefore, is represented by the sum of the 4 calculated percentages (Table 3, col. 3), while such a sum is likely to be too great in cases like that of map Ia. The fact that the sum of the calculated percentages was 9.22, while the actual percentage was 11, is due to the fact that the estimates are too small, owing to the correlation between p and d and also to the effect of random errors. Apparently, a correct minimum estimate for such conditions as those represented by map Ia would be given by the sum of the estimates at distance L and at distance 2.8 L.

The results of the application of the formula pd/4 to the data of tables 1 and 2 are shown in table 5. These results are, of course, valid only in case there has been no other cause of grouping in the orchards. They are comparable to results that would be produced by percentages of transmission, at distance L, ranging from 0.3 in orchard 5 to 8.9 in orchard 12. These percentages correspond to 2.2 and 17 per cent of the total number of dis-

eased trees in the two orchards. If the mode of transmission to trees at distances 1.4 L, 2 L, and 2.8 L is direct, as in maps I, II, III, and IV, the figures in table 5 are independent and should be summed up to give a minimum estimate of the total percentage of contaminated trees at the 4 distances. This will give 1.2 per cent of contaminated trees for orchard 5, and 20.9 per cent for orchard 12, corresponding to 8.7 per cent, and 39.8 per cent, respectively, of the total number of diseased trees.

If, on the contrary, transmission is partially or totally indirect, as in the case of map Ia, the figures relative to distances L and 1.4 L also contain the effect of the transmission to trees at the other distances, and these sums are likely to be too great. The figure for distance L does not, however, contain the effect of transmission to distance 2.8 L, and the sum of the figures for these two distances is apparently a good minimum estimate of the per-

TABLE 5.—*Minimum estimates of the percentages of "contaminated" trees, calculated by means of the formula pd/4,*[a] *from the data in tables 1 and 2, on the assumption that "diseased" trees were randomized at time of planting*

Orchard No.	Percentage of diseased trees[b] (p)	Calculations (pd/4) at distances:			
		L	1.4 L	2 L	2.8 L
4	10.3	0.5	—[c]	0.2	0.2
5	13.8	0.3	0.3	0.4	0.2
6	14.9	2.6	2.6	1.5	1.0
7	18.7	0.6	0.2	—	0.1
8	32.0	2.4	0.8	0.7	—
9	35.6	3.5	3.1	3.0	—
10	39.6	5.0	2.2	2.9	1.3
11	42.8	3.0	3.8	1.4	0.9
12	52.5	8.9	4.7	4.9	2.4
13	61.6	5.0	2.5	3.0	1.3
14	70.6	6.5	4.2	6.4	3.8

[a] p = percentage of diseased trees; d/4 = probability of contamination of any one of 4 adjacent trees by the central tree (see "Discussion").

[b] Based on the number of central trees for distance L.

[c] Dashes indicate negative differences for d, where diseased trees were greater around healthy trees than around diseased trees.

centage of transmission to the trees at the four distances, if no other cause of grouping occurs in the orchard. These totals range from 0.5 per cent in orchard 5 to 11.3 per cent in orchard 12, representing, respectively, 3.6 and 21.5 per cent of the total of diseased trees.

Among the possible causes of grouping of psorosis-affected trees, other than the transmission of the disease from diseased to healthy trees, is, as already mentioned, the grouping along rows by the planting of trees from the same parent trees. This would not affect the grouping along the diagonals, detected in the analysis for trees at distances 1.4 L and 2.8 L. For instance, the estimates of 0.2 per cent at distance 2.8 L for orchard 4 and of 4.2 per cent and 3.8 per cent at distances 1.4 L and 2.8 L, respectively, for orchard 14 (Table 5), are entirely independent of a possible grouping of trees at the time of planting. It was shown that in the case of only two orchards (see Table 1, orchards 4 and 14) did the analyses for diseased

trees at distance L from central trees, along rows and along arrays, show that such a grouping had possibly occurred. Figures for distances L and 1.4 L, in those two orchards, are apt to contain the effect of that kind of grouping.

Another cause of grouping would be the replacement of groups of psorosis-free trees by diseased trees. One possibility in this connection is the replacement of whole groups of trees that have died or declined as a result of waterlogging, or as a result of diseases to which waterlogging is a contributing condition, in parts of the orchard. Such a grouping, however, probably would be more plainly apparent on the maps than has been the case in the orchards used in this study.

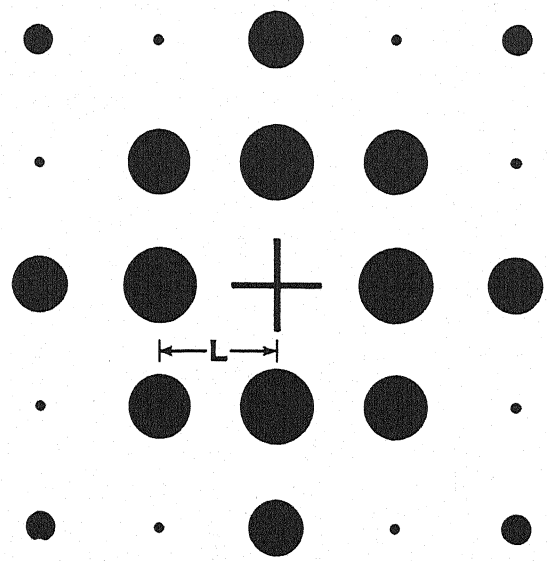


FIG. 3. Diagram illustrating the gradient of infectivity around a diseased tree. The diameters of the circles representing the 16 trees at distances L, 1.4 L, 2 L, and 2.8 L are proportional to the means of the differences in tables 1 and 2. The 8 trees represented as dots were not included in the study. These trees and others at greater distances, not shown in the diagram, would probably have shown small differences.

While the possibility of other causes of grouping cannot be dismissed, it does not seem probable that they would have acted in all the orchards used in this study, and it is safe to conclude that it has been demonstrated that a considerable percentage of trees, originally healthy in many orchards, may have contracted the disease from near-by trees that contained the virus at the time they were set out in the orchard.

There are several possible means of transmission that might be involved in the grouping of diseased trees revealed in this study; these include root-graft transmission or transmission by insects, rodents, or other animals, from tree to tree. The comparatively rapid decrease of the differences as the distance increases seems to favor transmission by root grafting. This decrease is actually a measure of a gradient of infectivity around diseased

trees. In the measuring of this gradient, all trees used as central trees are concerned; it is, therefore, the measure of a mean gradient of infectivity different from the gradient of infectivity often considered in problems of transmission of plant diseases in the field. Figure 3 represents this gradient

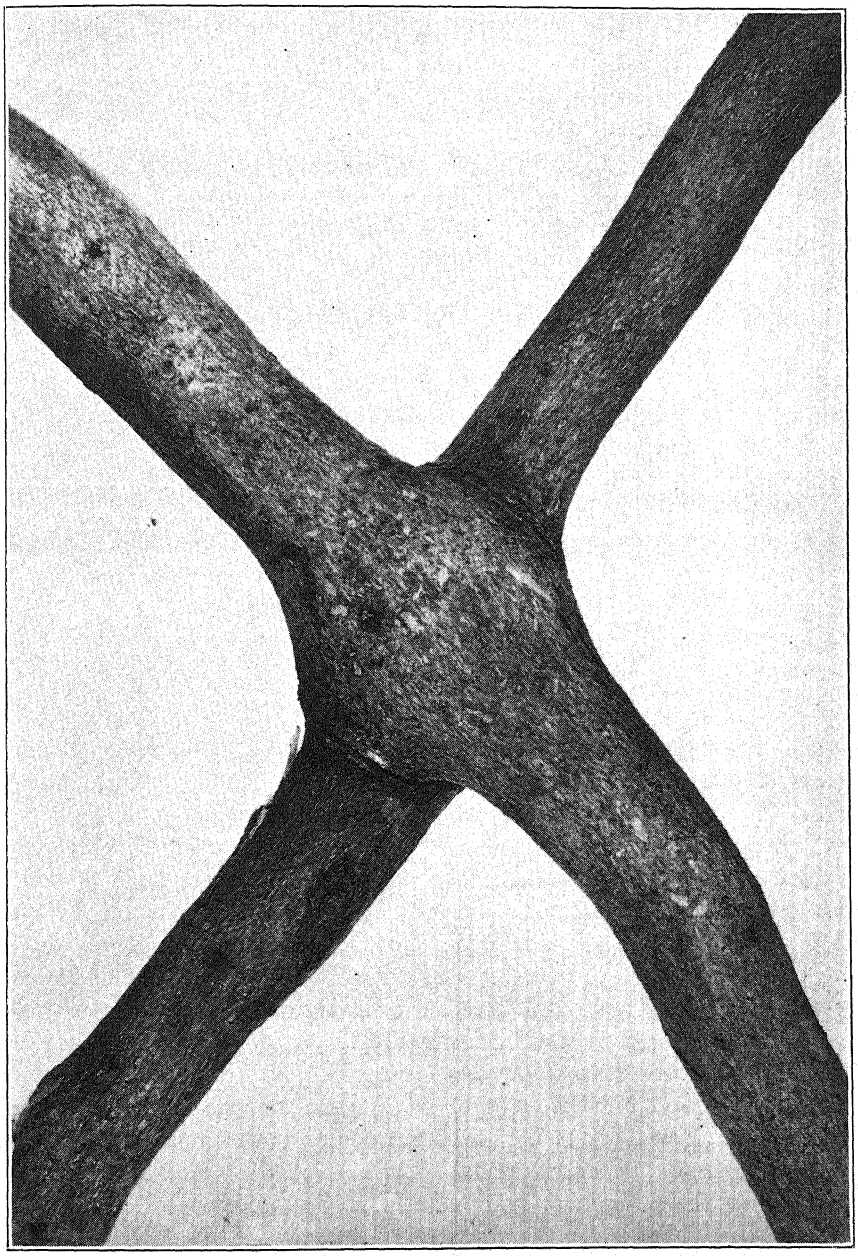


FIG. 4. Roots from adjoining trees, showing natural grafting where they had crossed. Such grafts have proved sufficient to transmit psorosis from one tree to another.

of infectivity diagrammatically. Each tree, except the central tree, is represented by a black circle, the diameter of which is proportional to the mean difference, at the given distance, for all the orchards showing a difference in favor of diseased central trees. This diagram shows that trees other than those considered in this study might have revealed transmission from the central tree if the method had been applied to them.

Observations have been made pointing to transmission by natural root grafting between roots of diseased and healthy trees. A number of natural root grafts have been found (Fig. 4) between adjacent trees in commercial orchards and between trees in the nursery. Root grafting has been shown to be a means of transmission by grafting experiments in which roots of diseased trees were grafted to healthy trees in the nursery and in the orchard. It is easily understood how a rather considerable number of new cases of psorosis may arise when the growth of the root system has reached the stage where natural root grafting is possible between adjacent trees. A number of healthy trees can be infected almost simultaneously if they are already connected by their root systems at the time one of them is naturally root-grafted to a diseased tree. Such a mode of transmission is comparable to the condition established in the preparation of map Ia.

Negative results only have been obtained in the limited number of experiments attempting transmission of psorosis by means of certain insects, and observational evidence appears to point to its not being an effective means in California. This means, however, cannot be entirely overlooked. Observational evidence, so far, suggests only a very slow and infrequent transmission from tree to tree by some such means, in addition to natural root grafting.

SUMMARY

On the maps of 14 sweet-orange orchards in California, on which psorosis-diseased trees, ranging from 3.8 to 70.6 per cent, had been recorded, counts of diseased trees around diseased trees and around healthy trees were made. The counts were made separately, first for the 4 trees closest to the diseased or healthy central trees (those at distance L from central trees), in all 14 orchards, and then, successively, for the 4 trees at distances 1.4 L, 2 L, and 2.8 L from central trees, in 11 of the orchards.

In one orchard there was a greater (nonsignificant) number of diseased trees at distance L from healthy trees than at distance L from diseased trees. In 13 orchards there were greater numbers of diseased trees at distance L from diseased trees than at the same distance from healthy trees, and the differences were significant or highly significant statistically in 11 of these 13 orchards. For these 11 orchards, differences in favor of diseased trees were found in 10, 10, and 9 orchards, at distances 1.4 L, 2 L, and 2.8 L, respectively, the differences being significant or highly significant in 7, 6, and 2 of the orchards for the respective distances. A marked decrease of the difference was observed as the distance from the central tree increased, the mean for all the differences available being 0.305 ± 0.022 for distance L,

0.262 ± 0.025 for distance 1.4 L, 0.233 ± 0.027 for distance 2 L, and 0.119 ± 0.027 for distance 2.8 L.

A separate analysis for the two trees at distance L from diseased trees and from healthy trees, along rows and along arrays, showed that in only two orchards were there significant differences in the mean number of diseased trees at distance L from diseased trees along rows and along arrays. This difference is interpreted as indicating that in those two orchards runs of diseased trees from the same budstick from diseased parent trees were set along rows at the time the orchards were planted.

Comparison with maps of fictitious orchards, on which given percentages of diseased trees were randomly distributed at given distances from randomly distributed diseased trees, showed that the differences found for the actual orchards were in some orchards comparable to those that would occur if from 2.2 to 17 per cent of the diseased trees in the orchard had become diseased as a result of transmission from diseased trees at distance L, with correspondingly decreasing percentages as the distance increased.

Among the possible causes of transmission of psorosis, root grafts occurring naturally in the orchard are considered as most likely to result in (1) the high percentage of transmission that would produce differences as great as those observed and in (2) the comparatively rapid decrease in the differences as the distance from a central tree increases. The possibility of additional means of transmission, as by insect vectors, cannot be entirely dismissed.

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CONTROL OF THE TRUFFLE IN BEDS OF THE CULTIVATED MUSHROOM

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The invasion of mushroom beds by the weed fungus, *Pseudobalsamia microspora* Diehl and Lambert, causes a substantial annual loss. The widespread fear of this organism among mushroom growers is principally due to their lack of knowledge concerning its source and control.

The truffle has been scientifically recognized since about 1927 (2). At the present time the organism is extremely widespread and probably exists, at least to some extent, wherever mushrooms are grown.

GENERAL DESCRIPTION OF THE INFESTATION

The fungus first manifests itself at the time of the first "break," or, later, as small wefts of white to cream-colored mycelium under the sideboards. About a week later the wefts become organized into the characteristic wrinkled, flattened "calves brains," or ascocarps (Fig. 1, C). At this stage ascocarps may begin to form over the surface of the soil and can be generally found in the compost directly underneath. The infestation may be restricted to several small plots in the bed or may affect the entire bed. Frequently, only a few beds in an entire house may show signs of it. Under some conditions only the top few inches of spawned compost may be invaded, although in other cases the invasion may be more general. The mushroom mycelium disappears in infested areas; the compost becomes black and damp, and mushroom production stops. A characteristic chlorine-like odor pervades badly infested houses.

The internally borne spores are formed shortly after the ascocarps appear and are liberated when the cream-white fruiting bodies become reddish-brown and punky, with subsequent disorganization. Ascocarps on the surface begin to break down after about 6 weeks, while those in the compost remain intact for a considerably longer time. Diehl and Lambert have thoroughly described the organism (2).

GERMINATION OF THE SPORES

Truffle spores may be germinated readily by inoculating a suspension into an ordinary bottle of pure-culture manure spawn. If desired the manure spawn may be autoclaved before inoculation with truffle spores, although there is no advantage in this. Wetting the spawn is unnecessary and may even lead to failure if done to excess.

The first evidence of growth is a luxuriant and conspicuous white mycelium around the top of the bottle (Fig. 1, A). Ascocarps form abundantly between the spawn and the glass walls of the bottle. Attempts to germinate spores on other media have failed. If sterile compost is simul-

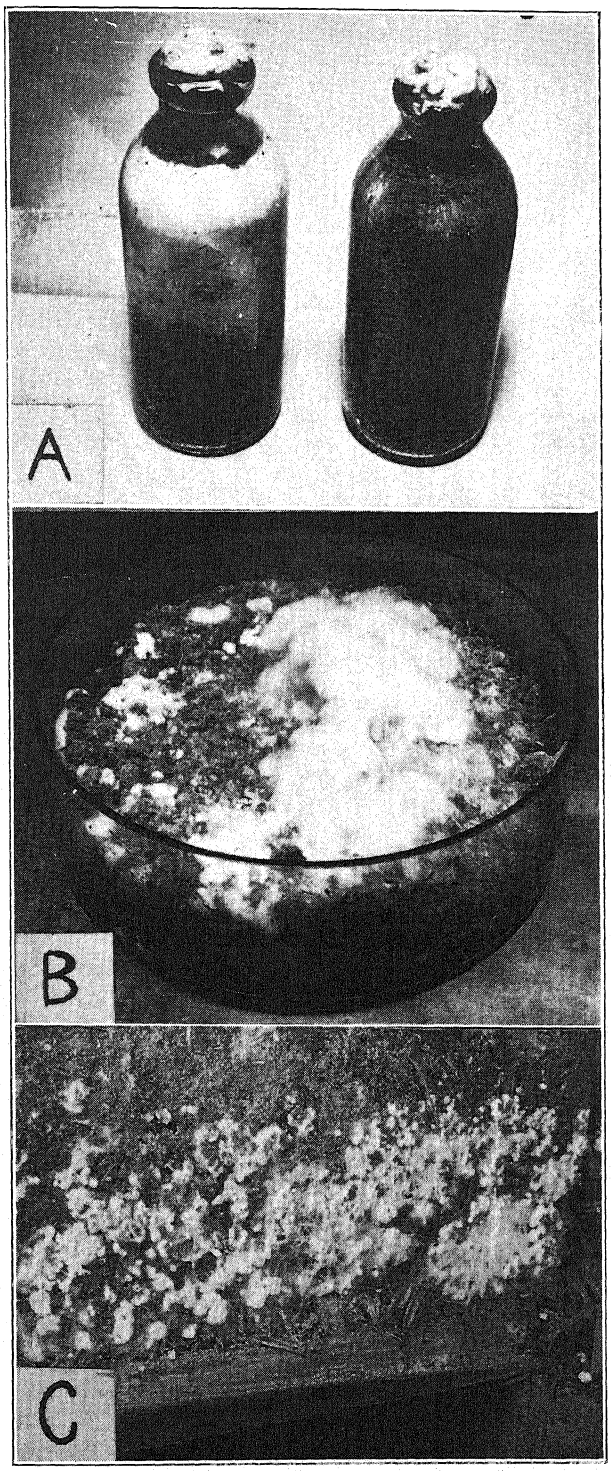


FIG. 1. Different aspects of the truffle fungus (*Pseudobalsamia microspora*) in culture. A. Mycelial stage. B. Thirty-day-old culture incubated at 74° F. C. Characteristic wrinkled ascocarp stage.

taneously inoculated with mushroom mycelium and truffle spores, the spawn will develop in the usual manner, but the subsequent appearance of the truffle is not at all conspicuous, and ascocarp production is reduced.

The optimum temperature for germination is 87° F. At this temperature growth becomes evident in about 11 days. At 60° F. the spores fail to germinate. At 72° F. an approximate incubation period of 25 days is required.

Germination in manure spawn occurs in the moisture range of 100–250 per cent on a dry weight basis. Spore germination shows a much more distinct preference for dry conditions than is commonly supposed. The optimum moisture condition for germination is about 130 per cent. Germination is retarded or may fail altogether when the moisture content exceeds 200 per cent.

FACTORS AFFECTING GROWTH

The optimum temperature for mycelial growth is 83° F. with a minimum at 52° F. and a maximum at 97° F. (1). The pH range in which growth is tolerated is 4.5–7.5, with an optimum at 6.8 (1).

In the temperature range 55–85° F., the truffle mycelium grows much more rapidly than that of the mushroom. When a Petri dish is simultaneously inoculated with both organisms and incubated within this temperature range, the mycelium of the truffle will virtually surround the mushroom mycelium in a short while.

Truffle mycelium grows in pure culture spawn in the moisture range of 100–300 per cent with optimum growth at 185 per cent on a dry weight basis.

CULTURE MEDIA

Pure-culture manure spawn is the most satisfactory medium for growth and ascocarp production. When pure-culture spawn is inoculated with truffle mycelium an abundance of fruiting bodies can be had in 9 or 10 days at 83° F. Potato-dextrose agar supports growth fairly well with limited production of ascocarps. The organism grows very weakly in sterile composted manure at pH 7 and not at all in nonsterile compost at the same pH. Growth on the former medium is considerably improved by the addition of a spawn extract. Growth is poor in ordinary garden soil, but, again, is rather good in soil taken from a bed that is producing mushrooms. It seems evident that mushroom mycelium releases a substance that is specifically stimulating to the growth of truffle. Good evidence that the truffle is not parasitic on mushroom mycelium is afforded by the fact that it will not grow in a rye spawn medium, even though mushroom mycelium flourishes on rye grains.

A rather good growth of truffle can be secured on oat hulls supplemented with garden soil. It grows well—even fruits—on moist mushroom-house boards, a factor to be taken into consideration when disinfection of the boards is contemplated.

SOURCE OF THE SPORES

At an early stage of his investigations, Lambert (4) suggested that the truffle probably inhabited the soil. Glasscock and Ware in England have brought forth evidence to support this view (3). Their observations on normal and infested beds cased with soils from different areas indicated that a particular soil source was carrying the spores. This presumptive evidence was confirmed when they succeeded in securing a growth of truffle by placing a layer of suspected soil over pure-culture spawn in a test tube and incubating at 83° F.

The following account implements the belief that the soil is the primary source of spores. A mushroom grower in Delaware converted a small stone structure near his main plant into a mushroom house. The manure, soil, and spawn used in this house and in the main plant were similar; nevertheless, every bed in the stone house was invaded by truffle, while the main plant was entirely free of the fungus. Further inquiry revealed that the manure for the small plant had been composted on a sodded area next to the regular composting ground. On Dec. 18, 1941, a number of soil samples were removed from the sodded area and placed over a 3-inch layer of manure spawn in preparation dishes 10 inches in diameter. The same procedure was followed with soil from the regular composting ground, as well as with garden soil used as a control. All dishes were incubated at 74° F. One month later the dishes containing soil from the sodded area showed a conspicuous growth of truffle (Fig. 1, B). The control and the soil from the regular composting ground were free of the fungus.

There can scarcely be any doubt that the sod was contaminated with truffle spores that were worked into the manure during the turnings of the heap. At least the upper inch of sod had been worked into the manure by this means. Later attempts to find ascocarps in the sodded area during the summer were unsuccessful. Incidentally, mushroom houses are as yet the only known habitat of the fungus.

The experience of another grower in the Oxford area is illustrative. Shortly after casing, the mycelium of the mushroom came up through the soil and formed a thin mycelial mat in several places throughout the house. Since the grower had none of the soil left with which he had originally cased his beds, he secured some nearby soil and distributed it lightly over the areas containing mycelial mats. Truffle later appeared in almost every one of these spots, whereas the rest of the house was not invaded. The house temperature was unusually high during this period and contributed to the severity of the outbreak. Again it seems very likely that the soil placed over the spots containing mycelial mats was contaminated.

Manure spawn often has been imputed as a possible carrier of spores. Distasteful as the idea is to manure-spawn makers, the possibility must be admitted. Unless the sterilization procedure is thorough, the heat-resistant spores might survive and be carried over into the beds when the spawn is planted. In bottles of compost contaminated with truffle spores before their

inoculation with mushroom mycelium, the appearance of truffle growth is not at all conspicuous, being limited to scanty ascocarp production with little evident mycelium. Since manure-spawn bottles are sold or put in storage after about 5 weeks of incubation, a small growth of this sort might go undetected.

In one particular instance a grower planted the two halves of his house with spawn from two different manufacturers. A large amount of truffle appeared in one half of the house but not at all in the other. All other factors in this house were identical. It would be difficult to avoid the conclusion that the spawn was contaminated.

The rapid spread of truffle in the mushroom industry after its recognition in 1927 might have been due to contaminated manure spawn. It has been the practice of certain spawn makers in the past to add soil to their compost; and the initial contamination of the raw material for spawn making might have occurred in this fashion. It should be emphasized, however, that the present spawn makers are keenly aware of the danger of contamination and have taken extraordinary steps to obviate it. There is very little likelihood that spawn purchased nowadays can contain living truffle spores. Since the truffle does not grow in rye spawn and there is little likelihood of rye grains being naturally contaminated, the possibility of spreading truffle spores through this medium must be discounted.

INFECTIVITY EXPERIMENTS

Beach (1) secured only occasional success when attempting to establish an infestation by inoculating the manure with spores.

In the winter of 1941 the writer made, without success, 16 separate attempts to infest mushroom beds. The spores were distributed in the compost before and after composting, after spawning, and also were applied to the casing soil. The inoculations were made in different houses in which the temperature and other conditions were regulated in a manner optimum for mushroom production. In none of these cases was the temperature favorable for the development of truffle.

On 5 occasions ascocarps that had appeared in a bed were deliberately removed and mixed with spawn of the same and different houses without resulting infection.

In another series of experiments small cans 9″ in diameter and about a foot high were filled with composted manure and placed in mushroom houses. These cans were inoculated with spores before and after the "sweat-out" and after casing. After the spawn had "run" in each can, 5 of these were removed to a room thermostatically controlled at 74° F. while the remaining ones were left in the mushroom house at about 62° F. After 6 weeks, 2 of the cans at 74° F. showed signs of truffle; neither the remaining cans at 74° F. nor those left in the house developed truffle within the next 2 months. Parts of this experiment were repeated with the invariable result that truffle did not always appear, even when the temperature was favorable.

In general, temperature appears to be a critical factor in the appearance of the weed fungus in contaminated beds; nevertheless, since growth does not always occur under favorable temperature conditions, other factors are obviously involved. This is a particularly complicating feature of the truffle problem. Further work on the factors influencing spore germination certainly is indicated. It should be pointed out that truffle spores always germinate in pure-culture manure spawn under favorable temperature conditions; hence, the answer to this problem lies in investigating the manure as it is prepared for mushroom growing.

On many occasions samples of spawned compost were taken out of mushroom houses and inoculated with truffle mycelium. In almost every case the mycelium was able to become established and form ascocarps. This was not the case when the same spawn samples were inoculated with spores. The failure to secure an infestation under apparently favorable conditions is not due to conditions adverse to mycelial development, but to conditions that suppress spore germination.

THERMAL DEATH POINT OF TRUFFLE SPORES

Spores have been reported (4) able to withstand a temperature of 180° F. for 5 hours. A series of bottles containing pure-culture spawn were inoculated with spores and subjected to various temperatures. The ability of spores to survive 180° F. for 5 hours was evident; however, they were killed after 7 hours at this temperature. Spores survived 3 hours at 200° F. In each case of survival at high temperatures there was no marked reduction of the incubation time. The spores are killed by the regular autoclaving procedure of 20 minutes at 250° F. The extraordinary resistance of the spores precludes any possible control by the use of heat.

FUNGICIDES

Stoller (5) exposed spores for 24–48-hour periods to 19 fumigants. Only ammonia, dimethylamine, quinoline, and methanol retarded germination for 4 months or longer, while the remainder were ineffective. Complete details of his tests were not given. The commonly used formaldehyde and sulphur dioxide gas were without effect. Apparently no fumigant gave a complete kill. Stoller suggests that the ammonia evolved during composting may serve to retard spore germination.

Fungicidal tests were made as follows: In the case of fumigants, ascocarps were crushed and laid upon glass slides to dry. The slide was then placed in a chamber saturated with each fumigant for the desired period of time. The spores were then washed off the slide and inoculated into duplicate bottles of pure-culture manure spawn. In the case of fungicidal liquids, the spores were suspended in water and 3 ml. of the suspension added to an equal volume of the liquid. After the desired exposure, the spores were filtered off under gentle suction on a thick asbestos pad. The pad was washed and the spores mixed into 50 ml. of water. Twenty-five ml.

of this asbestos suspension containing spores was put into each of 2 bottles of pure-culture manure spawn.

The spores were exposed for periods ranging from 1 to 6 hours. Various concentrations of liquid materials were employed. In no case were the spores killed. The following concentrations of various materials failed to kill after 6 hours: 1 per cent mercuric chloride, 2 per cent phenol, 2 per cent copper sulphate, 2 per cent ammonium hydroxide, 1 per cent glacial acetic acid, 1 per cent acetone, 1 per cent methyl alcohol, 0.50 per cent sodium ortho-di-nitro-cresolate, 0.50 per cent sodium tetra-chlorophenolate, 0.50 per cent sodium tri-chlor-phenolate. The following fumigants also were ineffective after 6 hours: formaldehyde, chlorine, sulphur dioxide, and chloropicrin. Although there was evidence of inhibition of germination in the cases of formaldehyde and ammonium hydroxide, a vigorous mycelium nevertheless appeared in the spawn bottles after 7 weeks.

CONTROL MEASURES

The soil is the primary source of spores: there are, however, various means by which the spores are actually introduced.

1. Infested soil may be added to the manure before or during composting. Many growers add soil because of the physical benefits conferred on the manure. Recently, it has become the practice to add 25–30 pounds of gypsum per ton of manure instead of soil, and this should be encouraged. It is not possible, apparently, to eradicate the spores in the soil by the use of steam or the usual fumigants.

2. The composting ground may be contaminated and spores may be worked into the manure during the turning of the heap. Composting over fresh sod or other plant-supporting ground should be avoided. Only a well-packed and well-drained composting ground should be used.

3. Spores may be brought in with contaminated casing soil. As mentioned above the ordinary techniques of soil sterilization are not effective against truffle spores. Under ordinary circumstances spores brought in with the casing soil are not likely to do any damage because the temperature usually is lowered sufficiently at this stage to prevent germination. Of course, this would not be true in unusually warm weather. In addition, soil-borne spores may serve to reinfest the following crop.

The failure of truffle spores to germinate at a temperature of 60° F. offers a positive means of controlling this weed fungus. At this temperature a perfectly normal, though slower, set of spawn can be secured. The loss in time is more than offset by lack of damage. An instance may be reported of a grower who suffered constantly from truffle until he was induced to run his spawn at a lower temperature, after which he became immediately free of it. Apparently, the question of reinfestation must be attacked in the same manner. Fungicides appear to be ineffective in eradicating the spores. Temperature control is, however, perfectly reliable and easy.

Experiment has shown that, even though truffle mycelium does grow on bed boards, the mycelium is easily killed by drying. There is probably very little trouble from this source.

Although the belief is widespread among mushroom growers that the truffle spreads from its primary site to other sites in the same or other beds, there is actually no evidence of this. Frequently only one or two spots of truffle appear in an entire house. The organism does not migrate through the compost continuously from a point of infection. Usually, it forms irregularly round patches and then stops. The universal appearance of the weed in the bed means that the spores were widespread initially.

As for possible bed-to-bed spread by means of spores, it should be pointed out that the temperature ordinarily is too low to permit their germination. Numerous experiments have confirmed this.

Growers commonly apply salt, formaldehyde, and other fungicidal materials to areas where ascocarps have appeared. This practice is not justified. In the first place the spores are resistant to the usual fungicides, and, even though they were not, it would be difficult to get at the internally borne spores, especially those contained in ascocarps deep in the manure. Secondly, by the time the application is made the infestation is already firmly established. Since secondary infection does not occur, the use of chemicals seems superfluous.

Truffle-infested areas frequently can be brought back into production if the area is not drenched with chemicals inimical to the mushroom spawn. Frequently, the invasion of the bed is superficial, involving only the top few inches of manure. In such cases there is usually healthy spawn underneath the infested area. Observation seems to indicate that the invasion tends to be superficial when the compost is packed rather tight in the bed. In such cases, the infested area can be dried up until the ascocarps have begun to desiccate and the truffle mycelium has passed its climax and disappeared. After this, water may be applied to encourage the spawn underlying the infested area to reinvade the compost and form fruiting bodies. The bed should be allowed to dry for 3 weeks or longer before it is watered again. One grower who has benefited greatly by this procedure estimates that he has been able to save about 50 per cent of his crop in infested areas. Of course when the invasion is deep and no healthy spawn remains in the bed it is futile to follow this procedure. The presence of spawn can be easily determined by inspection. The use of chemicals is not justified, even in the case of deep invasion.

The practice followed by some growers of digging a deep trench around infested areas also is not indicated, in view of the facts. It will, however, require considerable education to convince growers that the truffle does not spread from one place to another.

In removing an infested crop the grower should take particular care to dispose of the manure at a considerable distance from mushroom houses. It would be most desirable to be able to recommend a fungicide that could be

effectively used after removing an infested crop; unfortunately, no such material is known at this time. Consequently, the carry-over of spores from one crop to another is a most serious factor in the incidence of infestation. Again, temperature control must be relied on. Growers without refrigeration should by all means avoid late-spring or early-fall crops.

SUMMARY

Truffle spores were regularly germinated and ascocarps obtained by inoculating bottles of pure-culture manure spawn and incubating at 72°–85° F. Factors affecting spore germination and growth are described. Since truffle spores do not germinate at 60° F., a positive means of control is afforded through regulation of the temperature. Additional evidence is cited to show that the soil is the source of spores. In view of the fact that secondary infection does not occur, the practice of applying fungicides is not justified. When invasion of the bed is only superficial, production may be restored by drying up the infested area, followed by watering in the usual manner. A number of fungicides were found to be ineffective against truffle spores after six hours' exposure.

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FUSARIUM DISEASES OF BROAD BEAN. I. A WILT OF BROAD BEAN CAUSED BY FUSARIUM AVENACEUM VAR. FABAE N. VAR.[1]

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INTRODUCTION

The Fusarium wilt and foot rot of broad bean caused by *Fusarium avenaceum* (Fr.) Sacc. is the most destructive disease of this crop in Yunnan, China. During the past 4 years, considerable study has been devoted to the pathogenicity of this fungus; and, as was revealed, it comprises a number of physiological strains differing from one another in ability to induce either wilt or foot rot. In the inoculation experiments conducted during the past 3 years, the typical wilt-producing strain caused the characteristic wilt, and seldom, if ever, foot rot. As far as the writer is aware this is the first record of a plant wilt induced by *Fusarium avenaceum,* and, for the sake of clearness, it is assumed that we are concerned with a new variety of the species. This paper deals with the detailed description of this disease, with its distribution in so far as it is known, with experimental inoculations, and with description and identification of the fungus.

REVIEW OF LITERATURE

Four species of *Fusarium, i.e., F. gramineum, F. vasinfectum, F. avenaceum,* and *F. orthoceras* var. *pisi,* have been reported pathogenic to broad bean by various investigators. The first record of the Fusarium root rot of broad bean was provided by Kirchner (2) in 1890. Later, a wilt of broad bean, characterized by the slender shoots and few pods, was reported by Miyake (6) from Japan in 1924. Both Kirchner and Miyake found *Gibberella saubinettii* (*F. gramineum*) to be the causal agent.

In 1920, Pape (7) reported *Fusarium avenaceum* (*F. herbarum* var. *tubercularioides*) as causing a foot rot of broad bean. Wollenweber and Reinking (9) mentioned *F. avenaceum* f. 1 on the same host. Marchal and Verplanke (5) reported a wilt of broad bean in Belgium in 1926, and attributed the cause to *F. vasinfectum.* Linford (3), in connection with his investigation on the Fusarium wilts of peas in Wisconsin, found that *F. orthoceras* var. *pisi* is only weakly pathogenic to broad bean. Kadow and Jones (1) reported the occurrence of the pea-wilt organism on *Vicia gigantea* and Sutton's New Giant broad bean in Washington.

DISTRIBUTION OF THE DISEASE

The Fusarium wilt has been found well distributed over the important

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broad-bean growing sections of Yunnan. The first finding of the disease was at I-liang, in December, 1938. Subsequent examinations of plants from this same locality have shown it to be abundant. The gradually accumulating evidence from survey records shows it to be abundantly present in practically every section in this part of China. Many fields appeared to be completely infected.

ISOLATION OF THE FUNGUS

The basal stem portions of diseased plants were washed thoroughly in sterile water, then soaked in mercuric chloride solution (1:1000) for 20 minutes, and placed in Petri dishes in hard agar. Pure cultures were then made. The fungus may be obtained from the stem several inches above the soil surface.

SYMPTOMS OF THE DISEASE

The first symptom of the disease appears when the plants are near or at the blossoming stage. The leaves are first pale-green then light-yellow with black lesions on the leaf margin, especially on the top of the leaf. The upper leaves may be in an erect position, and may become more rigid than the normal ones. Sometimes the entire plant becomes yellow and the leaves wither progressively upwards to the terminal buds. The leaves also may become yellowish-green and quickly dried out. They are twisted, curly, and papery in texture; meanwhile, the stems turn black and wither. A plant may wilt within a week after the first symptom appears. In general, it takes 20 to 30 days to wilt completely under field conditions. Stunting of plants may be common with wilted plants.

Upon pulling any of the plants in the advanced stage of the disease it is found that they come out of the ground very easily, due to the fact that the root systems have been practically destroyed. Nearly all the fibrous roots are rotted off and the main root itself undergoes, in most cases, a dry decay. Vascular discoloration develops in the upper part of the tap-root and may extend into the stem to a considerable distance.

INOCULATION EXPERIMENTS

The ability of the fungus to infect the broad bean and produce the wilt typical of those plants from which the fungus has been isolated many times, has been established through inoculation experiments both in the field and under laboratory conditions. Infection takes place when a spore suspension of the fungus is added to the soil when the plants are several inches high.

On January 3, 1939, inoculations were made as follows: Twenty 60-inch-high broad-bean seedlings were grown in sterilized soil in pots with one plant per pot and inoculated by pouring a spore suspension on the basal stems. When examination was made on April 17, 1939, at which time the experiment was discontinued, wilt was found on 8 of the 20 plants. Check plants remained healthy. The fungus was isolated from these 8 plants and its identity determined.

On January 14, 1939, broad bean seeds were soaked for 30 minutes in mercuric chloride solution (1:1000), washed in sterile water, and planted in 12-inch pots containing sterilized soil at the rate of 5 seeds per pot. After these plants attained a height of 12 inches, 40 of them were inoculated with the fungus by pouring the spore suspension just on the base of the stems. Readings were made on April 29, 1939. Twenty-one of the 60 plants wilted. No foot rot was observed. The wilted plants were examined and the fungus was found growing on the roots causing browning discoloration of the vascular bundles on the tap roots.

In the fall of 1939, infection was obtained by inoculating seedlings of 3 varieties of broad bean. They were grown in 12-inch pots containing sterilized pure sand. The seeds were sown on October 19, 1939. The seedlings were inoculated 4 times at an interval of 20 days from November 20 of that year until January 19, 1940. The inocula were taken from cultures on steamed broad-bean stems. Heavy spore suspension was prepared. Infections were obtained in the months of March and April on all varieties and the checks were found to be sound. The percentages of infection were 38.2, 62.7 and 51.1, respectively.

Several other experiments similar to the above have been conducted at various times and have always yielded a high or low percentage of infection.

No evidence has been obtained that *Fusarium avenaceum* var. *fabae* is a cause of wilts on common bean, cowpea, tomato, wheat, oats, corn, carrot and pea.

DESCRIPTION AND IDENTIFICATION OF THE CAUSAL FUNGUS

Morphology. The following media were employed in the study and identification of the causal fungus: steamed rice, broad-bean stem and pod, locust stem and root, Sophora stem and root, Irish-potato plug, hard oat agar, hard potato-dextrose (2 per cent) agar, corn-meal agar, broad-bean-decoction agar (100 g. of seed per 1000 cc. distilled water), Czapek's agar, and 1 and 2 per cent dextrose standard nutrient agars. The agar media were in both test tubes and plates. The media were prepared according to the recommendations of Sherbakoff (8) and the committee on taxonomy of Fusarium (10).

In determining the size of macroconidia, at least 100 and sometimes 200 or more spores were measured. The measurements of them in various media are as follows:

Broad-bean stem; cultures 38 days old; macroconidia from sporodochia:
Macroconidia—
4-septate, 1 per cent, 45.6×4.4 μ.
5-septate, 99 per cent, 51.3×4.2 ($45.3–66.1 \times 3.5–4.7$) μ.

Broad-bean stem; culture 40 days old; macroconidia from sporodochia:
Macroconidia—
3-septate, 1 per cent, 34.8×3.5 μ.
4-septate, 13 per cent, 41.6×3.9 ($38.4–48.7 \times 3.5–3.8$) μ.
5-septate, 86 per cent, 46.3×3.5 ($38.3–55.7 \times 3.5–4.2$) μ.

Broad-bean-decoction agar; cultures 27 days old; macroconidia from sporodochia:
Macroconidia—
4-septate, 2 per cent, 56.8 × 3.8 (53.9–59.3 × 3.7–3.8) μ.
5-septate, 97 per cent, 58.6 × 3.7 (50.4–73.1 × 3.5–5.2) μ.
6-septate, 1 per cent, 73.1 × 4.5 μ.

Broad-bean-decoction agar plate; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 5 per cent, 50.8 × 3.7 (45.2–55.7 × 3.5–3.8) μ.
5-septate, 94 per cent, 57.0 × 3.7 (47.0–67.9 × 3.5–4.0) μ.
6-septate, 1 per cent, 62.4 × 3.8 μ.

Broad-bean-decoction agar plate; culture 40 days old, macroconidia from sporodochia:
Macroconidia—
5-septate, 95 per cent, 56.3 × 3.8 (43.5–67.9 × 3.5–4.4) μ.
6-septate, 5 per cent, 61.9 × 4.0 (53.9–67.9 × 3.7–4.5) μ.

Broad-bean-decoction agar plate; culture 44 days old, macroconidia from sporodochia:
Macroconidia—
5-septate, 98 per cent, 55.5 × 3.6 (48.7–67.9 × 3.5–3.7) μ.
6-septate, 2 per cent, 61.2 × 3.6 (60.9–62.6 × 3.5–3.7) μ.

Pea pod; culture 25 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 5 per cent, 44.2 × 3.7 (40.0–48.9 × 3.5–4.0) μ.
4-septate, 31 per cent, 47.7 × 3.5 (40.0–62.6 × 3.5–3.8) μ.
5-septate, 63 per cent, 53.9 × 3.5 (45.2–73.1 × 3.5–3.8) μ.
6-septate, 1 per cent, 66.1 × 3.8 μ.

Pea stem; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 4 per cent, 47.6 × 3.8 (45.2–55.7 × 3.7–4.0) μ.
5-septate, 94 per cent, 57.4 × 3.8 (43.5–67.9 × 3.5–4.0) μ.
6-septate, 2 per cent, 63.0 × 3.8 (64.4–69.6 × 3.7–4.0) μ.

Pea stem; culture 38 days old, macroconidia from sporodochia:
Macroconidia—
5-septate, 95 per cent, 56.2 × 4.4 (43.5–71.7 × 3.7–4.5) μ.
6-septate, 5 per cent, 63.3 × 4.0 (64.4–76.6 × 3.8–4.4) μ.

Pea stem; culture 40 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 2 per cent, 46.1 × 3.5 (45.2–47.0 × 3.5–3.7) μ.
4-septate, 7 per cent, 48.7 × 3.5 (41.7–55.7 × 3.5–3.7) μ.
5-septate, 90 per cent, 52.2 × 3.5 (43.5–67.9 × 3.5–3.7) μ.
6-septate, 1 per cent, 73.1 × 3.5 μ.

Sophora stem; culture 38 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 8 per cent, 48.7 × 3.7 (43.5–55.7 × 3.7–3.8) μ.
5-septate, 90 per cent, 51.9 × 3.7 (45.2–59.2 × 3.7–3.8) μ.
6-septate, 2 per cent, 73.1 × 3.8 μ.

Locust stem; culture 43 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 1 per cent, 51.5 × 3.7 μ.
5-septate, 91 per cent, 61.8 × 3.6 (48.7–73.1 × 3.5–4.0) μ.
6-septate, 8 per cent, 70.3 × 3.7 (62.6–74.8 × 3.5–4.0) μ.

Locust root; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 4 per cent, 53.6 × 3.6 (50.1–59.2 × 3.5–3.8) μ.
5-septate, 96 per cent, 57.4 × 3.6 (48.7–66.1 × 3.5–3.8) μ.

Locust root; culture 43 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 10 per cent, 47.9 × 3.6 (38.8–52.2 × 3.5–3.8) μ.
4-septate, 31 per cent, 50.8 × 3.6 (40.0–55.7 × 3.5–3.7) μ.
5-septate, 59 per cent, 56.0 × 3.7 (48.7–62.6 × 3.5–3.8) μ.

Locust root; culture 45 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 5 per cent, 53.6 × 3.7 (48.7–60.9 × 3.5–3.7) μ.
5-septate, 95 per cent, 48.5 × 3.7 (48.7–69.6 × 3.5–3.8) μ.

Potato plug; culture 44 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 3 per cent, 46.5 × 3.6 (43.5–48.7 × 3.5–3.7) μ.
5-septate, 95 per cent, 52.9 × 3.6 (43.6–64.4 × 3.5–3.8) μ.
6-septate, 2 per cent, 56.6 × 3.6 (50.5–62.6 × 3.5–3.7) μ.

Hard oat agar plate; culture 30 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 2 per cent, 42.8 × 3.5 (33.1–50.5 × 3.8–4.0) μ.
4-septate, 8 per cent, 42.1 × 3.8 (40.0–59.2 × 3.7–3.8) μ.
5-septate, 88 per cent, 54.4 × 3.8 (47.0–73.1 × 3.5–4.2) μ.
6-septate, 2 per cent, 64.0 × 4.0 (62.6–67.9 × 3.8–4.2) μ.

Hard oat agar tube; culture 43 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 3 per cent, 56.7 × 3.6 (55.7–57.4 × 3.5–3.7) μ.
5-septate, 90 per cent, 63.0 × 3.7 (52.5–71.7 × 3.5–3.7) μ.
6-septate, 6 per cent, 69.6 × 3.6 (60.9–73.1 × 3.5–3.7) μ.
7-septate, 1 per cent, 73.1 × 3.7 μ.

Czapek's agar plate; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 7 per cent, 40.0 × 3.7 (31.3–45.2 × 3.7–3.8) μ.
4-septate, 23 per cent, 42.2 × 3.6 (38.3–52.2 × 3.5–3.8) μ.
5-septate, 70 per cent, 49.1 × 3.7 (38.3–57.4 × 3.5–3.8) μ.

Averages:
3-septate, 1.47 per cent, 42.8 × 3.6 (34.8–47.9 × 3.5–3.8) μ.
4-septate, 7.98 per cent, 49.4 × 3.7 (41.6–56.7 × 3.5–4.4) μ.
5-septate, 88.53 per cent, 55.4 × 3.8 (46.3–63.7 × 3.5–4.2) μ.
6-septate, 0.05 per cent, 73.1 × 3.7 μ.

The measurements of 200 macroconidia taken from the root of an artificially infested plant are as follows:

Macroconidia from sporodochia—
3-septate, 12.5 per cent, 39.2 × 4.4 (44.1–48.7 × 3.7–5.2) μ.
4-septate, 10 per cent, 47.7 × 4.7 (36.5–53.9 × 3.8–5.2) μ.
5-septate, 75 per cent, 56.7 × 4.7 (41.8–69.6 × 3.8–5.2) μ.
6-septate, 2 per cent, 65.7 × 4.8 (60.9–69.6 × 3.8–5.2) μ.

From the foregoing spore measurements, it is seen that the macroconidia produced in sporodochia on cultural media and on the infected plants agree fairly well as to spore size and the distribution of the spore septation, with 5-septate being the predominant. The diameters of spores from diseased plants are, however, greater than those of spores produced in culture media.

The pseudopinnotal spores, on the other hand, vary greatly in shape and size, as well as in number of septations. For example: certain measurements of macroconidia taken from pseudopinnotes are given below:

Standard nutrient agar tube (1% dextrose); culture 20 days old, macroconidia from pseudopinnote:
Macroconidia—
3-septate, 16 per cent, 31.8 × 3.8 (27.8– 34.8 × 3.7–4.2) μ.
4-septate, 4 per cent, 47.9 × 3.9 (45.2– 50.1 × 3.7–4.2) μ.
5-septate, 18 per cent, 49.1 × 4.0 (40.6– 59.2 × 3.7–4.4) μ.
6-septate, 14 per cent, 58.4 × 4.4 (48.7– 67.9 × 3.8–4.2) μ.
7-septate, 22 per cent, 67.5 × 4.1 (55.7– 76.6 × 3.7–4.2) μ.
8-septate, 10 per cent, 76.6 × 4.7 (66.1– 83.5 × 3.7–4.2) μ.
9-septate, 12 per cent, 76.6 × 4.7 (69.6– 87.0 × 3.7–4.2) μ.
10-septate, 2 per cent, 92.2 × 4.7 (83.5–100.9 × 4.2–5.2) μ.

Standard nutrient agar tube (2% dextrose); culture 20 days old, macroconidia from pseudopinnote:
Macroconidia—
0-septate, 6 per cent, 19.1 × 3.6 (18.1–20.9 × 3.5–3.7) μ.

1-septate, 28 per cent, 19.4 × 3.6 (15.7–22.6 × 3.5–4.5) μ.
2-septate, 24 per cent, 24.1 × 3.7 (19.1–31.3 × 3.5–4.4) μ.
3-septate, 32 per cent, 35.0 × 3.6 (20.9–38.3 × 3.8–4.2) μ.
4-septate, 4 per cent, 40.0 × 3.7 (38.3–41.7 × 3.7–3.8) μ.
5-septate, 6 per cent, 45.8 × 3.7 (43.5–50.5 × 3.5–3.8) μ.

Hard oat agar plate; culture 20 days old, macroconidia from sporodochia:

Macroconidia—
1-septate, 2 per cent, 27.8 × 3.8 μ.
2-septate, 2 per cent, 24.4 × 3.8 μ.
3-septate, 8 per cent, 30.5 × 4.4 (24.4–48.7 × 3.5–5.2) μ.
4-septate, 16 per cent, 37.2 × 4.2 (31.3–48.7 × 3.7–5.0) μ.
5-septate, 30 per cent, 46.9 × 4.3 (36.5–66.1 × 3.7–4.8) μ.
6-septate, 16 per cent, 57.9 × 3.9 (45.2–69.6 × 3.7–4.8) μ.
7-septate, 18 per cent, 71.9 × 4.4 (67.9–79.0 × 3.5–5.2) μ.
8-septate, 2 per cent, 83.2 × 3.9 μ.
9-septate, 2 per cent, 92.2 × 3.9 μ.
10-septate, 0 per cent.
11-septate, 2 per cent, 102.6 × 3.9 μ.
12-septate, 2 per cent, 107.4 × 3.9 μ.

CULTURAL CHARACTERS

Rice: Twenty-five-day-old cultures are white, pink, amber-yellow, forming a medium-dense and matted mass of mycelium over the rice (4). The mycelium may be white, pink, ivory, or lilac below. Sclerotia are produced in great abundance.

Broad-bean-decoction agar: Twenty-five-day-old cultures bear a scant, thin, loose and white mycelial growth. Rufous-orange sporodochia are formed in great abundance.

Corn-meal agar: Twenty-five-day-old cultures bear a scant, thin, white, cream, pinard-yellow or chrome-lemon-yellow aerial growth.

Potato-plug: One-month-old cultures show a compact, dense, thick or matted growth of mycelium that is white, Jasper-pink, wild-rose or ash-rose. The surface of the medium is wrinkled, and powdery in appearance. At the base and the side of the slant, the color of the mycelium is marsh-rose, pepper-red and slate.

Hard oat agar: One-month-old cultures form a dense, thick, matted, white, lilac, Tango-pink, leather to buttercup-yellow and France-rose mycelial growth. The mycelium may be powdery in places. Holly-berry-red to hyacinth-red is seen in the substratum. Mycelium attached to wall at base of slant is lilac, Grecian-rose, slate, rufous-salmon, and primrose-yellow. Sporodochia small, slimy, spherical to subspherical, solitary or aggregated, and rufous-orange. Pseudopinnotes mandarin to rufous-orange.

Czapek's agar: One-month-old cultures are characterized by dense, compact, somewhat matted, pink to raspberry-red mycelium. When spores are produced in abundance, culture is rufous-salmon, slimy, and conspicuously matted.

Standard nutrient agar (plus 1 or 2 per cent dextrose): One-month-old cultures form abundant, compact, dense, and pinkish mycelial growth with powdery mycelial masses. Chimney-red, pink, lilac and slate lines are seen at the base of the culture.

Broad-bean pod: One-month-old cultures are characterized by dense, compact, white, coral to candy-pink or raspberry-red powdery mycelial growth. Rufous-orange and slimy sporodochia of various sizes are produced.

Broad-bean stem: Young cultures are characterized by an extremely fine, fluffy, and cottony-white mycelial growth. In old cultures, there is an abundant, more or less compact, cottony, white, bluish-rose, light- to sulphine-yellow growth with small, cream-color powdery masses in places on the mycelium. Sporodochia are found over the stems, in places on the mycelium, or even on the moist cotton at the bottom of the tubes.

Pea pod: One-month-old cultures form abundant, slightly compact to long, loose cottony, matted, white, cream, pink, spruce and pyrite-yellow mycelial growth with cream-color powdery masses in places on the mycelium.

Pea stem: One-month-old cultures show thin, cottony, matted, white, blush-rose, sulphine-yellow mycelial growth with cream to light-rose powdery masses in places on mycelium. Sporodochia are produced over stem, in places on mycelium, or even on moist cotton at base of tube.

Locust stem: One-month-old growth is scant, loose, thin, cottony, white, and pink. Sporodochia are produced in places on mycelium, over twigs, or on moist cotton.

Locust root: When one-month-old, scant, white mycelial growth with cream-color powdery masses in places on the mycelium are produced.

Sophora stem: One-month-old cultures form moderate growth of loose or compact, thin, cottony, white and blush-rose mycelial growth usually powdery in appearance.

Sophora root: At first the aerial mycelium is white, very scant, but fine and cottony. In two-month-old cultures still scant, white in places, usually light-blush-red.

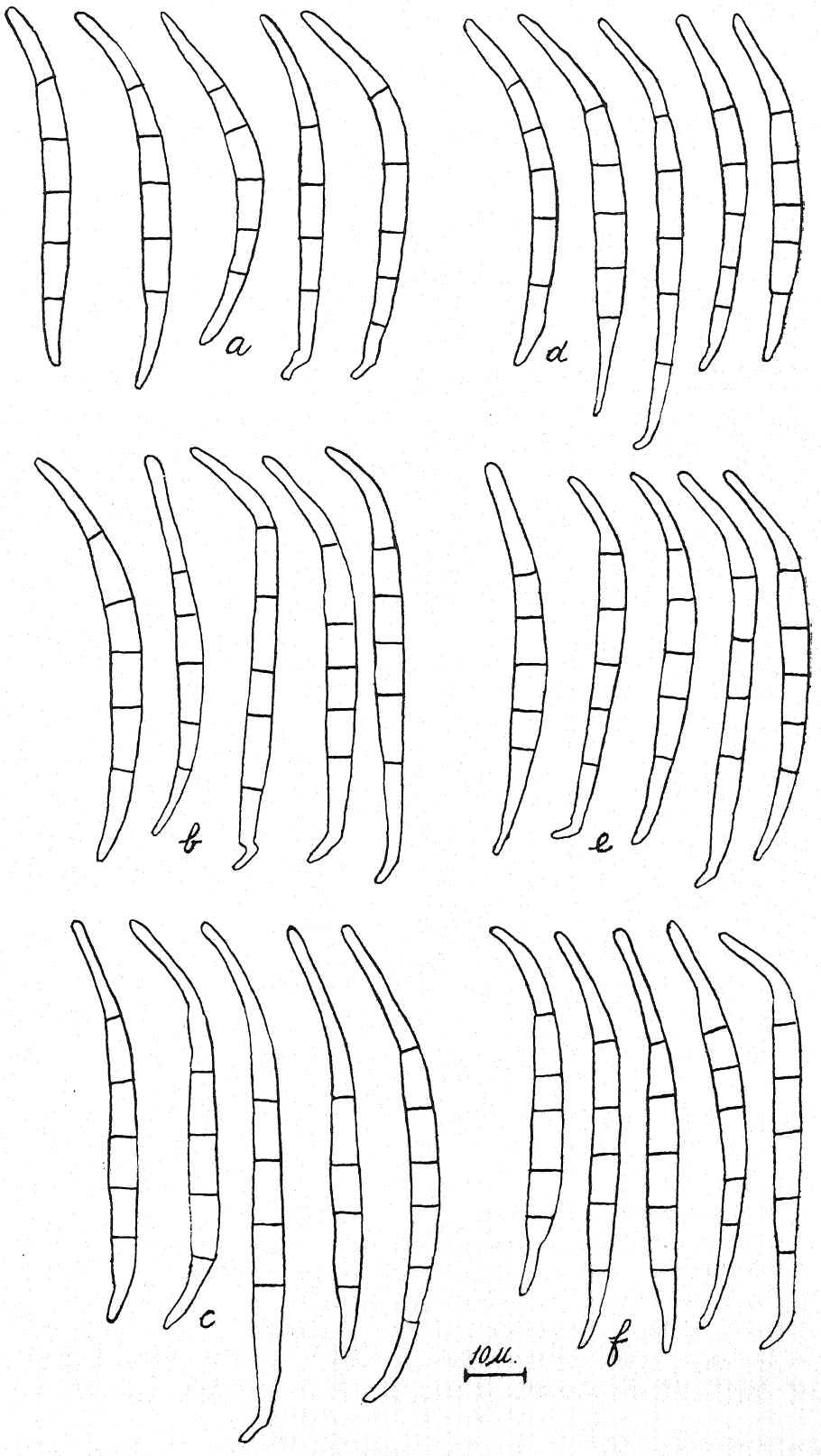


FIG. 1. Macroconidia of *Fusarium avenaceum* var. *fabae:* (a), from 37-day-old culture of steamed broad-bean stem; (b), from 30-day-old culture of broad-bean-decoction agar tube; (c), from 30-day-old culture of locust stem; (d), from 30-day-old culture of hard oat agar tube; (e), from 30-day-old culture of pea stem; and (f), from 30-day-old culture of locust stem.

Potato-dextrose (2 per cent) agar plate: One-month-old plates form a medium dense, thick, loose or matted pinkish mycelial growth over the surface. The mycelium in the substratum is Isabella, and absinth-yellow. Spots of Bokhara to cyclamen-purple with tourmaline-pink outlines are seen.

Hard oat agar plate: One-month-old cultures form matted, compact and white to blush-rose mycelial growth. In the substratum, the mycelium is raspberry to blush-rose with ruby or garnet, zonate.

Corn-meal agar plate: Mycelial growth of 1-month-old cultures, thin, loose, cottony and white to pink. The mycelium in the substratum is Isabella, absinth-yellow with few zones. Mycelial knot seen as spot of Bokhara-purple, cyclamen, with pinkish outline scattered in the plate. The knot, when examined microscopically, shows heavy and thin hyphae clamping together to form knots of various sizes.

Broad-bean agar plate: In 1-month-old culture, the aerial mycelium absent or scant. When present, only few white, thin mycelial tufts scattered in the plate. Sporodochia are, in general, produced in great abundance in this medium.

Czapek's agar plate: One-month-old cultures form thick, matted, compact and white to pinkish aerial mycelium. In the substratum, mycelium is sultan- to ruby-red, zonate.

Standard nutrient agar plate (1 or 2 per cent dextrose): One-month-old cultures have an abundant, loose or compact, matted and white to blush-rose mycelial growth. In the substratum, the mycelium is buttercup-yellow to buff and raspberry to blush-rose with crimson, mineral-red, ruby-red, cardinal-red or slate zones.

In general, sporodochia are readily produced on many kinds of media. They may be produced over the media, in places on the mycelium or even on the cotton at the bottom of the tubes. Pseudopinnotes are less frequently produced. Microconidia are only rarely seen. Chlamydospores have not been found in any of the media tested. Sclerotia are produced abundantly on steamed rice and to a lesser extent on potato plug. The stromatic structures are occasionally found.

The morphologic characters (Fig. 1) of the fungus, as determined from cultures of various media, are as follows:

Microconidia absent, or rarely produced, oval, oblong or kidney-shaped; 0–1-septate, 0-septate, 10.7×3.7 ($8.7–13.9 \times 3.5–3.4$) μ; 1-septate, 13.9×3.4 ($10.4–15.7 \times 3.3–3.4$) μ. Macroconidia in sporodochia, curved-spindle-shape, awl-shape, thread-like, or anguliform, long, comparatively narrow and slender, broader in middle, with long end cells, the cells detached when old, often slender, pointed and in most cases sharply bent, pedicellate or more or less 4-celled, typically 5-septate, 55.4×3.8 ($46.4–63.0 \times 3.5–4.2$) μ, rarely 7-septate, exceptionally up to 12-septate; 3-septate, 42.8×3.6 ($34.8–47.9 \times 3.5–3.8$) μ; 4-septate, 49.4×3.7 ($41.6–56.7 \times 3.5–4.4$) μ; 6-septate, 62.9×3.8 ($56.6–73.1 \times 3.5–4.5$) μ; 7-septate, 73.1×3.7 μ. Macroconidia pseudopinnate varying greatly in size, shape and number of septation; from 0–12-septate. Sporodochia spherical or irregular in outline, often fluid or slimy, shiny and rufous-orange. Pseudopinnotes less frequently produced, spreading, slimy, shiny and rufous-orange. Sclerotia, deep blue-black, rough, oval, round or irregular in size, large 1.4 mm. in diameter (0.2–2.5 mm.). Chlamydospores, absent.

From a comparison with descriptions given by Wollenweber and Reinking (9), it is evident that this fungus is in the section Roseum of the genus *Fusarium*, and within that group lies close to *Fusarium avenaceum*. It differs from *F. avenaceum*, *F. avenaceum* forma 1, *F. avenaceum* var. *pallens* and *F. avenaceum* var. *volutum* (9) in its ability to produce a typical wilt of broad-bean and is, accordingly, considered as a new variety of this species.

SUMMARY

A wilt disease of broad bean caused by *Fusarium avenaceum* var. **fabae** var. nov., is herein described.

The disease is known to occur in the important bean-growing sections of Yunnan, China, where it causes considerable damage to the crop.

The leaves of the diseased plants first turn greenish-yellow, then wither, and the plants eventually die. The vascular regions of the infected plants, especially the tap root and basal stem, are shaded brown to dark-brown.

The causal fungus of this disease is described as *Fusarium avenaceum* var. *fabae*. Its morphology and cultural characters are described.

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STUDIES ON BACTERIAL CANKER OF TOMATO

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INTRODUCTION

Bacterial canker of tomato caused by *Phytomonas michiganensis* (E.F.S.) Bergey *et al.*, 1923, is the major bacterial disease of field-grown tomatoes in California. Although the disease is known to be seed-borne, the literature on the biology and related features of the pathogen is very limited. This paper presents some additional data on certain aspects of this disease.

VARIANTS

Pink and white strains of *Phytomonas michiganensis* have been reported (3, 4, 5). The white strain was obtained from the yellow (normal) strain and is stable under laboratory conditions, while the pink strain is unstable, yielding both white and yellow strains (5). The white strain is pathogenic to tomato, while the pink strain is only slightly pathogenic, or, in many instances, non-virulent (5). The writer has studied both the white and the pink strains of the organism and compared them with several isolations of the yellow or normal strain.

The white strain was first obtained in the laboratory as a variant of the yellow strain when the latter was grown in a beef-extract-peptone agar medium for 2 months. Pure culture studies and pathogenicity tests showed the white strain to be *Phytomonas michiganensis*. The physiologic studies were made with cultures that were passed 3 times through tomato plants. There were some differences between the white and the yellow strains when the organisms were grown in SAB synthetic medium (12) for 6 weeks. Thus, the white strain produced some gas in galactose, glycerine, levulose, and lactose in 6 weeks, while no trace of gas was observed for the yellow strain. Also, the white strain was twice isolated from field material in association with the yellow strain.

Two Strains

A pink strain of *Phytomonas michiganensis* was obtained from an infected tomato and the white-rough strain from spontaneous dissociation in a test tube. The colonies of the pink strain were small, delicate pink, glistening, and spread out with age. The slant growth was pink with a yellowish hue. The rough strain was similar to the white (smooth) strain in its behavior as to sugars in SAB synthetic media. On potato-dextrose(2%)-peptone(1%)-agar(2%) plates, colonies began to appear after 4 days' incubation at 28° C., and at first were minute, gradually becoming raised and wrinkled over the surface of the colony. En masse growth on slants is not slimy but rather dry, soon showing numerous small folds imparting a

wrinkled appearance. The growth was not stringy, but compact, becoming flocculent in saline solution (0.85% NaCl). The culture retained its characteristics on an artificial laboratory medium (beef-peptone agar) for a year. On potato plugs it gave smooth colonies after a month's growth. In pathogenicity tests, all the variants studied were pathogenic to tomato plants, but in varying degree. The white-smooth and white-rough strains were pathogenic in about the same degree and did not form open cankers on tomato stems. These two strains caused slight wilting of the leaflets, or of the whole leaf, and darkening of the cambial and vascular region. Plants inoculated with these strains grew to maturity and produced fruits. Numerous unsuccessful attempts were made to isolate the organisms of these strains from tomato fruits of plants that had previously been artificially inoculated. The pink strain is less pathogenic than the white or white-rough strain. Green tomato fruits, previously treated for 2 minutes with a 1:1000 solution of corrosive sublimate, washed in sterile distilled water, and then sprayed with the pink strain, showed minute, dark-brown to black spots that, on culturing, yielded the pink strain.

HOST RANGE

Phytomonas michiganensis is reported only on tomato, except for the statement of E. F. Smith (11) on the possible infection of potatoes. The red currant tomato, *Lycopersicum pimpinellifolium* Mill., is only slightly susceptible to the tomato canker organism, infection being slow and of a mild type, *i.e.*, cankers are not readily formed and the plant is not killed. The symptoms consist of a discoloration of the cortex and vascular region. Attempts to isolate the organism from fruits of the artificially infected plants were unsuccessful. Tree tomato (*Cyphomandra betacea* Sendt) is stunted and shows a black discoloration in the fibrovascular system after artificial inoculation with *P. michiganensis,* but never develops cankers nor does it die. *Solanum nigrum* L. var. *guineense* L., behaves similarly when inoculated.

Tobacco (*Nicotiana tabacum* L.) failed to become infected, while *N. glutinosa* L. was readily infected and the organism recovered. Yellowing and wilting of the leaves occurred and occasionally very small cankers developed on the stems.

TESTS WITH INSECTS

The insects reported below were reared in the greenhouse on healthy, muslin-covered plants most favorable for their development. Generally, 25 to 50 insects were transferred to diseased tomato plants from which transfers of 5 to 15 insects were later caged on healthy young tomato seedlings in individual pots previously kept insect-free by frequent fumigation. Infested plants were held under observation for 2 months. The disease failed to develop in any case where contaminated insects were used. Furthermore, the canker organism was not isolated from the mouth parts or the internal organs of the insects that had fed on diseased plants. The following insects were tested: green peach aphid (*Myzus persicae* Sulz.), tarnished plant bug

(*Lygus pratensis* L.), onion thrips (*Thrips tabaci* Lind.), and the twelve-spotted cucumber beetle (*Diabrotica duodecimpunctata* Fabr.).

INFECTION BY THE TOPPING KNIFE

By cutting off the tips of young tomato seedlings with a contaminated knife a higher percentage of infection results than does from the needle-prick method of inoculation.

Fifty young, thrifty tomato plants (lot 1) were inoculated by needle pricks and 50 plants (lot 2) by cutting off the tips with a sharp dissecting knife, previously contaminated with the organism from agar slants. The plants were kept under observation for 30 days. All the plants in lot 2 became infected, while only 4 per cent of those in lot 1 contracted the disease.

In another test, 30 vigorous, healthy tomato plants, consecutively numbered, were grown in individual pots. A diseased plant was cut with a cartilage knife so as to expose the entire blade to diseased tissue. The tips of the 30 plants were then cut off with the contaminated knife in a manner simulating commercial practice, resulting in 30 distinct cases of bacterial canker that clearly originated at the cut surface. Another series of 47 plants were cut with a similarly contaminated knife, and 37 developed distinct cankers originating at the cut surface.

Three flats of healthy tomato plants, containing a total of 104 plants, were cut with a knife contaminated by cutting a diseased plant. Twenty-nine out of 33 plants in flat 1, 32 out of 33 in flat 2, and 11 out of 38 in flat 3 developed the disease. This indicated that canker can be carried to numerous plants on the knife once it becomes contaminated.

Healing of the knife cut is a slow process, as shown by the following greenhouse experiments. One lot of 125 greenhouse-grown tomato plants, averaging 6 inches in height, was divided into 5 groups. The growing points of all plants were decapitated with a very sharp knife. One group of 25 plants was untreated and served as checks for the other 4 groups. All plants in the remaining group were smeared on the cut surface with a heavy paste of *Phytomonas michiganensis* at the following intervals after wounding: 24, 48, 72, and 96 hours. The final reading was made in the greenhouse after 30 days. All of the plants smeared with *P. michiganensis* 24 hours to 72, inclusive, after wounding, became infected. No disease developed on any plants smeared 96 hours after wounding.

INFECTION BY HANDLING

Fifty young plants were handled as a grower would do when pulling plants from a plant bed for transplanting. First, a diseased plant was pulled rather roughly, and then the same treatment was given to 50 consecutive plants numbered from 61 to 110. Of these, 66, 78, 80 and 83 developed canker. This demonstrated that healthy plants may be infected by the hands during the operation of transplanting.

These data indicate two ways in which the disease may be spread under commercial field conditions, and are in agreement with the observations of Müllers (9) and others.[1]

ROOT INOCULATIONS

The writer's experiments with root inoculations of tomato plants gave negative results. One hundred tomato plants, grown in individual pots, which had had their roots cut either by a contaminated knife or a clean knife followed by pouring a heavy suspension of *Phytomonas michiganensis* into the soil around the injured roots, remained healthy. This is not in accordance with the statement of Blood (2) that "wounded roots appear to be highly favorable infection courts," nor with the experiments of Orth (10).

ATOMIZER INOCULATION

Attempts to induce the disease in young tomato plants kept in moist chamber for 24 hours prior to spraying with a heavy suspension of *Phytomonas michiganensis* in distilled water were unsuccessful. Perhaps, because of the non-motility of the tomato-canker organism, the stomata are apparently unsatisfactory infection courts.

EFFECT OF GERMICIDAL TREATMENTS ON THE ORGANISM

The effect of various concentrations of alcohol, brilliant and malachite green, and rosaniline hydrochloride dyes on the organism and on tomato seed have been investigated. The dyes were dissolved in water and in alcohol of different strengths. In preliminary tests, potato-dextrose-peptone agar was prepared and enough alcohol added to make 5, 10, 15, 20, 25, 30, 35, and 40 per cent. The alcohol was added aseptically after melting the agar. The medium was then poured into Petri dishes and, after solidification, streaked with a heavy suspension of *Phytomonas michiganensis*. Control plates were without alcohol. All plates were incubated at 28° C. No growth was observed on any of the alcohol plates. Control plates developed good growth in 2 days.

Similarly, a dye agar containing 1:1000 and 1:2000 concentrations of brilliant and malachite green and rosaniline hydrochloride was prepared. The dyes and medium were sterilized simultaneously. No growth of *Phytomonas michiganensis* occurred in any of these concentrations after 10 days. Tests were then conducted to determine how long it takes each of these solutions to kill the tomato-canker organism (Table 1). Two cc. of each solution was used in agglutination tubes. A loopful of an agar-slant growth of the canker organism was introduced into a test tube, with the test solution, and vigorously shaken. Streaks were made on potato-dextrose-peptone agar plates at intervals of 5, 10, 15, and 60 minutes. All plates were incubated at 28° C. for 2 weeks and records taken once every 2 days (Table

[1] The writer is indebted to Professor J. B. Kendrick, Division of Plant Pathology, University of California, Davis, for some of the data on transmission of the disease by the contaminated knife.

TABLE 1.—*Effect of alcohol and 1 to 1000 alcoholic and water solutions of dyes on Phytomonas michiganensis in water suspension*

Treatment	5% alcohol					30% alcohol					Water (H_2O)				
	Exposure in minutes and hours														
	5	10	15	60	24 h.	5	10	15	60	24 h.	5	10	15	60	24 h.
Malachite green	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brilliant green	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosaniline hydrochloride	+	+	+	+	0	0	0	0	0	0	+	+	+	0	0
Control	+	+	+	+	+	0	0	0	0	0	+	+	+	+	+

+ = organisms grew upon streaking.
0 = no growth after streaking.

1). It is interesting to note that the addition of alcohol to the dye somewhat lowers its germicidal effect (Table 1). Thus, while a water solution (1:1000) of brilliant green and malachite green killed the organism in 5 minutes, it required 10 and 15 minutes, respectively, when 5 per cent alcohol was added.

EFFECT OF GERMICIDES ON SEED GERMINABILITY

The effect of the above-mentioned materials on tomato seed also was investigated (Table 2). The same procedure was used, except that the seeds were thoroughly washed in distilled water immediately after treatment and subsequently placed on slightly moist filter paper in Petri dishes for germination at 28° C. Tomato seed was not injured by the concentrations effective in killing the tomato-canker organism, as shown in table 2.

EFFECT OF HEAT ON GERMINABILITY OF THE SEED

The writer also studied the effect of dry heat (relatively high temperatures) on the germination of tomato seed (var. Santa Clara Canner). In experiments on the viability of tomato seeds exposed to dry heat, Miss Jozefowicz found (6, 7) that seeds withstood dry heat up to 79.5° C., even

TABLE 2.—*Effect of 1 to 1000 alcoholic and water solutions of dyes on the germination of tomato seed*

Treatment	5% alcohol					30% alcohol					Distilled water				
	Exposure in minutes and hours														
	5	10	15	60	24 h.	5	10	15	60	24 h.	5	10	15	60	24 h.
Malachite green	4+	4+	4+	3+	+	4+	4+	4+	3+	0	4+	4+	4+	4+	4+
Brilliant green	4+	4+	4+	4+	+	4+	4+	4+	2+	0	4+	4+	4+	4+	4+
Rosaniline hydrochloride	4+	4+	4+	4+	+	4+	4+	4+	3+	0	4+	4+	4+	4+	4+
Control	4+	4+	4+	4+	+	3+	3+	3+	3+	0	4+	4+	4+	4+	4+

+ = very slow germination.
2+ = slow germination.
3+ = slightly retarded germination.
4+ = normal germination.
0 = no germination.

TABLE 3.—*Effect of dry heat on germination of tomato seed*

Temperature, °C.	Control	50 50 55 55 60 60 65 65 70 70 75 75 80 80 80 85 85 95
Exposure, hours	.....	24 48 24 48 24 48 24 48 24 48 24 48 1 10 24 1 15 1
Per cent germination	80	61 65 84 23 64 74 73 10 51 12 40 55 53 50 51 60 40 0

for 72 hours, "the germination being more or less retarded, according to the temperature and time of exposure."

The results shown in table 3 show that tomato seed (2 years old) can withstand prolonged dry-heat treatment.

Dried on the surface of cover slips, the organism can remain alive for 2 hours at room temperature and for 10 minutes at 50° C.

Tomato seed[2] obtained by crushing ripe fruits from canker-diseased plants and subsequently by air-drying and storing at room temperature in the laboratory, for several months, were subjected to different treatments as indicated in table 4. Seeds treated with chemicals were afterwards washed in distilled water and then germinated in flats of steam-sterilized soil. The young plants, with 2 or more true leaves, were transplanted into large boxes of steamed soil, using about 500 plants of each lot (Table 4).

The canker organism is spread by the topping knife and by handling during transplanting. Thus, a few diseased plants may serve as a focus of infection, since a knife contaminated by cutting through or handling one diseased tomato plant is capable of infecting at least 30 healthy plants. The hot water treatment at a temperature of 53° C., effective in killing the tomato-canker bacteria, is known also to be injurious to the seed (10).

The writer's experiments with alcohol, alcoholic and aqueous solutions of malachite green, brilliant green, rosaniline hydrochloride, and dry heat show that the bacteria may be successfully eliminated by these methods. The writer noticed no ill effects on tomatoes in field plantings of either alcohol or dry-heat-treated seed. No field data on malachite, brilliant green, or rosaniline solutions are available, but greenhouse tests revealed no injury on plants after such treatments.

TABLE 4.—*Percentage of cankered tomato plants grown from treated and untreated seed collected from diseased plants*

Treatment		Percentage of plants diseased
Material and concentration	Duration	
Brilliant green 1: 1000 in water	60 minutes	2.9
Brilliant green 1: 1000 in 30% alcohol	60 minutes	1.5
Malachite green 1: 1000 in water	60 "	4.3
Malachite green 1: 1000 in 30% alcohol	60 "	0.7
Alcohol. 30%	60 "	0.0
Check (contaminated, untreated)	.....	26.0
60–61° C. (dry heat)	24 hours	0.0
65–68° C. (dry heat)	24 "	0.0

[2] Professor J. B. Kendrick kindly supplied the writer with some highly contaminated seed during the course of these studies.

SUMMARY

White, pink and rough variants of *Phytomonas michiganensis* (E.F.S.) Bergey *et al.* were studied and compared with the normal strain, physiologically and pathogenically. The variants are slightly different from the normal strain in their reactions on media and are less pathogenic.

Nicotiana glutinosa L. and *Cyphomandra betacea* Sendt., were successfully infected.

The following insects, *Myzus persicae* Sulz., *Lygus pratensis* L., *Thrips tabaci* Lind., *Heliothrips fasciatus* Perg., and *Diabrotica duodecimpunctata* Fabr., did not transmit the disease under greenhouse conditions. *Phytomonas michiganensis* could not be recovered from their mouth parts and internal organs after feeding on diseased plants.

Higher percentages of infection were obtained when the plants were topped with a contaminated knife than when punctured with a contaminated needle. The knife cuts remain susceptible to invasion 72 hours.

Unwounded plants did not develop the disease when sprayed with a heavy suspension of *P. michiganensis* and incubated in a moist chamber.

Brilliant and malachite green dyes are effective in killing the pathogen of tomato canker in aqueous and alcoholic solutions. Soaking tomato seed for more than 60 minutes in 5 per cent and 30 per cent alcoholic solutions of brilliant green, malachite green, and rosaniline hydrochloride, as well as in water solutions of these dyes, is not injurious.

Dry heat did not injure tomato seeds exposed to temperatures high enough to kill the pathogen. Dry seeds can withstand air temperatures up to 85° C. for 15 hours. This, however, reduced germination.

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GREENHOUSE METHOD FOR TESTING DUST SEED TREATMENTS TO CONTROL CERTAIN CEREAL SMUTS[1]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY,
COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

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The principal causes of variability in the results obtained at times by different workers in cereal smut control with the same chemical dusts may be summarized as follows:

1. Relation of host and pathogen; differences in the relative susceptibility of the varieties or the virulence of the smut fungus strains used.
2. Method of inoculation; the spore dosage employed and the method of applying the inoculum to the seed (8, 9, 10).
3. Application of fungicide; amount of seed used, type of apparatus for applying the dust, thoroughness of application, and the period and conditions of storing the treated seed (5).
4. Environmental conditions between sowing and emergence; depth of sowing and compactness, moisture content, temperature, and reaction of the soil (1, 2, 7, 8, 12, 13, 16).
5. Post emergence conditions; this phase has not been entirely worked out but it has been shown that smut development in cereals may be influenced by certain conditions after emergence, chiefly temperature and perhaps moisture and length of day. It is thought these conditions may affect the development of the fungus until after it reaches and is established in the growing point of the host plant (7, 17, 18, 19).

The following procedures for use in greenhouse studies have been prepared primarily for testing commercial materials that have been placed on the market and also promising new fungicides upon which sufficient preliminary research has been done by the originators. They are not intended for use in developing new fungicides except as a final test of these in comparison with standard materials already on the market. In the earlier stages of developing new fungicides, the technique must necessarily be modified to meet the varying conditions.

BUNT OF WHEAT (TILLETIA TRITICI (BJERK.) WINT. AND T. LEVIS KÜHN)

Inoculum. Use spores from fully mature bunted heads collected from the crop of the current season; in dry areas spores from the previous crop may be substituted. Dry the bunt balls thoroughly and then break them up by rubbing through a 20-mesh sieve, followed by a series of finer sieves, the last one being 60-mesh. Keep this inoculum in a loosely stoppered bottle or jar at 5° to 10° C. until it is to be used.

Host. Use a highly bunt-susceptible variety of spring wheat, along with a physiologic race of the bunt organism especially pathogenic to it. The variety Ulka (C.I. 11478) is susceptible to almost all physiologic races and *Tilletia levis* race 3 is widely distributed; accordingly, these are recommended as standard.[2]

Inoculation. In order to inoculate the wheat, use a spore dosage of 1 to 200, *i.e.*, 5 grams of 60-mesh spore material to 1,000 grams of clean seed (136 grams of smut per bushel). Shake the seed, with the inoculum applied, in a tightly closed, half-filled container for one minute, by which time the seed should be thoroughly blackened. Sift the seed lightly to remove any excess spores.

Application of Fungicide. If the seed sample used in testing dust fungicides is too small, much of the proportionately smaller amount of chemical dust will adhere to the

[1] Reprints may be obtained at 10 cents each from the Committee Chairman, Boyce Thompson Institute, Yonkers 3, New York.

[2] A limited supply of seed grain and inoculum may be obtained through the courtesy of the following investigators:
Ulka wheat—R. H. Bamberg, Montana Agr. Exp. Station, Bozeman, Montana.
Anthony oats—M. B. Moore, Agr. Exp. Station, University Farm, St. Paul, Minn.
Odessa barley—C. S. Holton, Agr. Exp. Station, Pullman, Washington.
Odessa barley—V. F. Tapke, Bureau of Plant Industry Station, Beltsville, Md.
Tilletia levis, race 3—C. S. Holton.
Ustilago avenae—C. S. Holton.
Ustilago hordei, race 6, and *U. nigra,* race 4—V. F. Tapke.

container in which the treating is being done. Therefore, the container should first be "conditioned" by treating in it a sample of any kind of clean wheat, which can then be discarded, leaving the inside of the container coated with the fungicide to be tested. The size of the seed sample to be employed usually is governed by the quantity of available seed and the number of materials to be tested. A sample of 500 cc., *i.e.*, approximately 1/70 of a bushel, is recommended as a standard. This sample should be taken volumetrically by using a 500-cc. container. If the fungicide is to be applied at the rate of 1/2 ounce per bushel, a 500-cc. sample will require 0.2 gram (1/70 of 14.17 grams). Rates of 1, 2, 3, and 4 ounces per bushel will therefore call for 0.4, 0.8, 1.2, and 1.6 grams, respectively. This simplifies calculations involving bushel weights of different crops and also avoids a slight error in the rate of applying dust fungicides to samples of light, chaffy seed and plump, heavy seed of the same crop. The container should be large enough to hold more than twice the quantity of seed used so that it will be less than half-filled. After the sample of seed has been placed in the container, the proper quantity of fungicide, carefully calculated and weighed, is added and the container, with a dust-tight cover, is given a thorough shaking for two minutes. The treated seed is then placed in a cloth sack or open container and stored in a dry place for 48 hours when the amount necessary for greenhouse planting is removed. The remainder of the seed may be kept for later germination tests, or for testing further, under field conditions, particularly those materials eliminated in the greenhouse tests. Such field tests are advisable, however, only in areas where spring wheat is adapted.

Planting the Seed. Plant a portion of the treated seed 1¾" deep in flats of soil, 50 per cent water saturated and with a reaction between pH 5.5 and 7.5 (14). A minimum of 5 replications of 100 seeds each for each test is recommended. Tamp the soil slightly after planting to bring it in close contact with the seed (1). Place the flats at 10° C. until after emergence and then remove them to a greenhouse held at 15° to 18° C. until the beginning of stem elongation or "shooting," when the temperature may be raised to about 25° C. If necessary, electric lights should be used to produce a photoperiod equal to that generally associated with the growing of spring wheat. If space at 10° C. is limited, the seed may be more thickly planted in smaller containers and, after emergence, the seedlings may be transplanted to flats or to the greenhouse bench. It goes without saying that in all such tests untreated, similarly inoculated seed should be planted under the same conditions for comparison.

Recording Results. Take data on emergence before the second leaf stage and on bunt infection at the stage when the diseased heads are most readily recognized. Percentages of infection should be based on head counts only. The significance of differences should be determined by statistical analyses; usually the analysis of variance (15) will be suitable for this purpose. In some cases transformation of data and cumulative error terms may be used to advantage (11).

LOOSE SMUT (USTILAGO AVENAE (PERS.) JENS.)

Inoculum. Since the two smuts of oats (*Ustilago avenae* and *U. levis*) are similar in life history and amenability to control by fungicides, the use of one species in testing dust fungicides is considered sufficient. Loose smut (*U. avenae*) is recommended as standard because of greater ease in preparing the inoculum.[3] Use spores from fully matured heads collected from the crop of the current season. Care must be taken to collect the heads of loose smut as soon as they appear and before the smut has been blown away. Sift the smut through a series of sieves, the last one being 60-mesh. Store the smut at 5° to 10° C. in a loosely stoppered glass container until it is to be used.

Host. Use the highly smut-susceptible spring variety Anthony (C.I. 2143).[4] This variety is recommended as a standard because, although not widely grown, it is susceptible to all commonly occurring physiologic races of *Ustilago avenae* and, therefore, the use of a particular race is not necessary.

Inoculation. Investigators have demonstrated that the smuts of oats may be perpetuated by spores on the seed, by spores and mycelium under the hull, and by mycelium in the epidermal cells of the pericarp (3, 6, 9, 12, 13). The infection due to spores on the seed is most easily prevented by seed treatment (9) while that due to inoculum under the hull is less amenable to control. It seems, therefore, that if a fungicide is to be thoroughly tested for oat smut control, it should be used on seed carrying inoculum under the hull (9). The use of a standard spore suspension and a partial vacuum in inoculating the seed is, therefore, recommended (4, 9, 10). In order to make a spore suspension, add 2 grams of 60-mesh spore material to a liter of distilled water and shake thoroughly until the spores are in suspension. Immerse 500 cc. of clean oats in one liter of this suspension in a high pressure desiccator and stir until the oats are thoroughly wet. To insure all kernels being beneath the liquid, a wire screen that fits the inside of the

[3] See footnote 2.
[4] See footnote 2.

vessel should be placed on the surface of the liquid. A wood strip an inch thick is fastened to its upper side. Install a vacuum gauge in the suction line and apply 25 inches of vacuum for 5 minutes, shaking the jar occasionally to promote the escape of air from beneath the glumes. After 5 minutes, release the vacuum and then apply it again for another 5 minutes. After again releasing the vacuum, drain the seed and spread it out on absorbent paper to dry for 24 hours to allow air to get under the glumes. Then place the seed in a one-half inch layer at 20° C. and 80 to 90 per cent humidity for 20 hours, after which it should be dried thoroughly with a fan and stored in a dry place until it is to be used.

Treating, Planting. The directions for treating and planting the seed are similar to those given for wheat, except that the temperature between planting and emergence should be from 18° to 20° C., the soil reaction should be about pH 7.4 (13), and the soil should be about 30 per cent saturated.

Recording Results. Data on emergence and on the percentage of smut are taken as described for wheat. Frequently, complete control of oat smuts in vacuum-inoculated seed is not obtained, even with dust fungicides that eliminate smut completely in naturally infested seed (9). In such cases the fungicidal efficiency of new materials being tested can be determined by comparing their performance with that of a standard fungicide known to be effective, and included in the same test.

COVERED SMUT (USTILAGO HORDEI (PERS.) KELL. AND SW.) AND BLACK OR SHALLOW-BORNE LOOSE SMUT (USTILAGO NIGRA TAPKE) OF BARLEY

Inoculum. The most prevalent and widely distributed physiologic races of the above smuts in the United States are race 6 of *Ustilago hordei* (16) and race 4 of *U. nigra* (21). Therefore, the use of these races is suggested as part of the standard procedure in testing dust fungicides for the control of these two barley smuts.[5] The inoculum should be collected and prepared for use as described for the smuts of wheat and oats.

Host. Odessa barley (C.I. 934) is highly susceptible to all known races of covered smut (16) and the shallow-borne loose smut (21). Therefore, this variety is recommended as a standard. It should be noted that this is not the commercially grown Odessa but the special strain labeled C.I. 934. This strain is highly susceptible also to the deep-borne loose smut, *Ustilago nuda,* which can be controlled only by the hot-water treatment. Because the heads of these two loose smuts closely resemble each other, seed free from *U. nuda* infection must be used to avoid confusion. Since the extreme susceptibility of Odessa (C.I. 934) makes it undesirable commercially, seed must be obtained from a station where increase plots are grown to maintain a soure of supply for experimental purposes.[6]

Inoculation. The inoculum of barley covered smut that is most effective in producing the disease and also most difficult to combat is that found beneath the hulls (20). Smut spores borne on the surface of the seed may be eliminated by copper carbonate, a fungicide that is generally considered unsatisfactory for controlling naturally induced covered smut of barley. Therefore, as in the case of the oat smuts, in order to test adequately a fungicide for the control of barley covered smut, seed should be used that carries abundant inoculum beneath the hulls. The same probably holds true for black loose smut (*Ustilago nigra*). The method of inoculating barley seed with either of the two smuts is the same as that described for the oat smuts.

Treating, Planting, Results. The treating and planting of the seed and the recording of the data are also similar to those for the oat smuts except that, for covered smut, the soil reaction may range from pH 6 to pH 7 (2) and for black loose smut the range appears to be still greater (7). A soil reaction of pH 6.5 and a temperature of 20° C. during emergence are suggested for both of these smuts.

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SEED TRANSMISSION OF SQUASH-MOSAIC VIRUS[1]

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INTRODUCTION

There are several virus diseases common to squash, *Cucurbita pepo* L. var. *condensa* Bailey, along the coast and in the inland districts of southern California. Of these virus maladies, western cucumber mosaic and squash mosaic are the ones most frequently observed. This paper is concerned with squash mosaic.

The disease occurs in the Tijuana Valley on the Mexican border and has been observed in the San Francisco Bay area, but is perhaps most often found in the coastal districts, from San Diego to Santa Maria. Though not so commonly observed inland, squash mosaic has been found in the western portions of Riverside and San Bernardino counties, but not as yet in the further inland desert areas of the Coachella and Imperial valleys.

In southern California, squash mosaic is present, but is relatively unimportant early in the growing season. As the plants begin to mature fruit, the disease may be found more frequently. Toward the close of the growing season it is omnipresent and responsible for considerable loss to the grower. In the San Francisco Bay area, however, squash mosaic is very important early in the season, especially in fields near the foothills; it has been suggested that the incidence of early infection may be closely related to the occurrence of overwintering forms of *Diabrotica* spp., which may serve as a source of the virus.

Kendrick (8) described a mosaic disease of muskmelons, *Cucumis melo* L., and proved the disease to be seed-transmitted. Infection percentage varied from 0.0 to 2.13, with a mean percentage infection of 0.25. Freitag[2] has identified the virus with which Kendrick worked as the squash-mosaic virus affecting muskmelons. Freitag (6, 7), in two abstracts dealing with squash mosaic, presents the properties of the virus, establishes insect transmission by the western spotted cucumber beetle, *Diabrotica soror* Lec., the western striped cucumber beetle, *D. trivittata* (Mann.), and 5 species of aphids. He found that the disease could be produced by inoculation in 15 species of plants distributed in 11 genera within 4 families.

Few references reporting seed transmission of cucurbit viruses are to be found in the literature. McClintock (10) indicates that cucumber-mosaic virus may be seed-borne. Doolittle and Gilbert (4) report seed transmission of the cucumber-mosaic virus by the wild cucumber, *Echinocystis lobata* (Michx.) Torr. and Gray. Doolittle (3) concludes that only one plant out of 10,000 grown from seed collected from infected fruits devel-

[1] Paper No. 502, University of California Citrus Experiment Station, Riverside, California.

[2] In correspondence with the writer, March 22, 1943.

oped mosaic symptoms not attributable to outside infection. He further states that "it is doubtful whether cucumber mosaic is seed-borne to any considerable extent, but it seems possible that it may occur in rare cases, and the results so far obtained have left the problem still open, warranting further investigation." Doolittle and Walker (5) present data showing one case of apparent mosaic seedling infection in approximately 22,000 cucumber plants; negative results were secured with mosaic-infected squash, muskmelon, and pumpkin. Bewley and Corbett (2) believe the cucumber-mosaic virus to be readily seed-transmitted. Mahoney (9) presents evidence of seed transmission of an unestablished virus in inbred lines of muskmelons; infection percentage varied from 14.3 to 33.3 and averaged 15.6. Ainsworth (1) states that yellow-mottle mosaic of cucumber, *Cucumis sativus* L., has

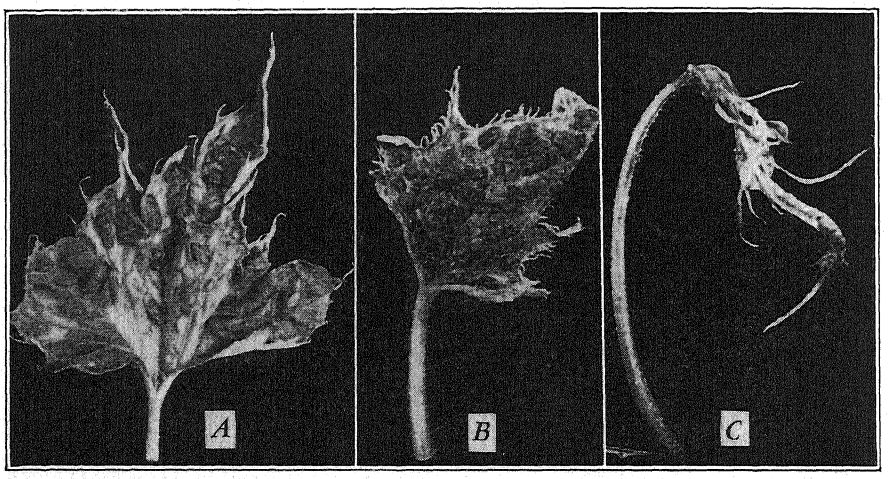


FIG. 1. Leaves of yellow crookneck squash, showing symptoms produced by the squash-mosaic virus. *A*. Distorted, rugose, mottled condition typical of the disease. *B*. Extreme rugosity. *C*. Leaf structure reduced largely to veinous system.

been shown to be seed-transmitted and believes that the yellow-mosaic and green-mosaic viruses are also seed-transmitted, but he gives no supporting data.

SYMPTOMATOLOGY

The most conspicuous and spectacular symptom of squash mosaic is the presence of filiform leaves; infected leaves are frequently reduced to only the veinous system, with patches of green mesophyll (Fig. 1). Leaves may be distorted, rugose, and mottled with dark-green raised areas; little if any yellowing occurs. Fruits from infected vines are malformed, the usual even contour broken by raised, more or less domelike areas, $\frac{1}{4}$ to $\frac{1}{2}$ inch in diameter; very often the affected areas are yellow or mottled (Fig. 2). Infected plants fail to grow with normal rapidity, set fewer fruits than healthy plants, yet rarely die as a direct result of squash-mosaic infection.

SEED SELECTION AND PLANTING

In order to determine whether or not the squash-mosaic virus is seed-transmitted, mature fruits showing typical fruit symptoms were collected from badly diseased vines. Collections were made in San Diego and Orange counties. Three varieties were chosen: White Bush Scallop, Yellow Crookneck, and Italian Marrow or Zuchini. Seeds were extracted by scooping them out of the fruits and washing thoroughly in water. Many of the infertile and light seeds were floated off during the washing. The remaining

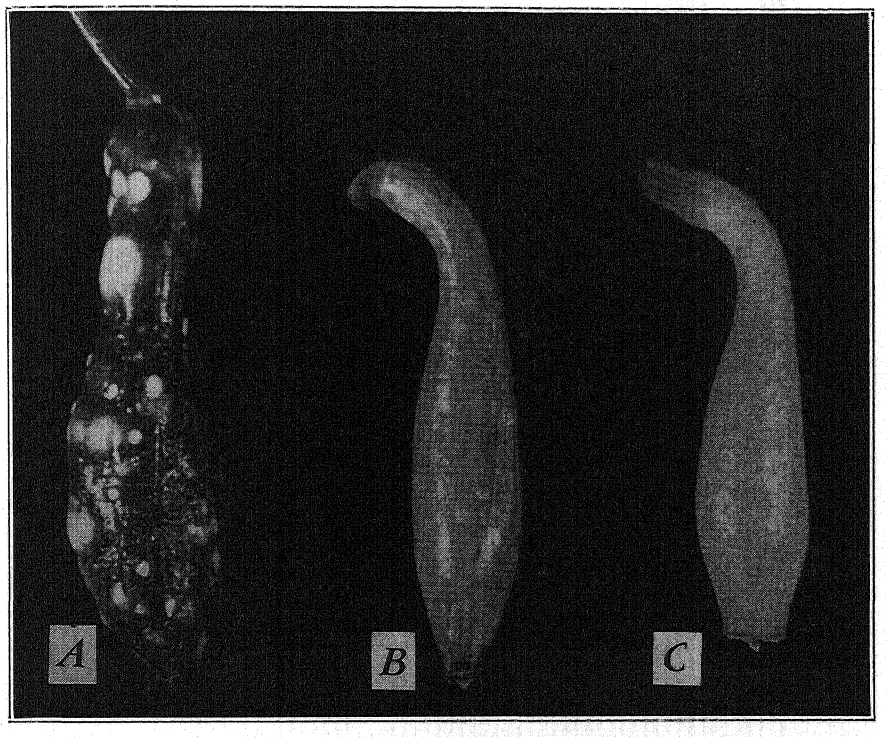


FIG. 2. Immature fruits of yellow crookneck squash, showing effect of infection by the squash-mosaic virus. *A*. Severe symptoms. *B*. Mild symptoms. *C*. Healthy fruit.

seeds were then dried and winnowed, and the light, poorly filled, deformed seeds were segregated from the heavy, well-filled seeds.

Seeds were sown in sterilized soil contained in sterilized redwood seed flats, which were placed on benches in a glasshouse. Separate sowings were made of the light, poorly filled, deformed seed and of the heavy, plump seed. Plantings were made on different dates, at similar intervals, over a period of about 3 years. The section of the glasshouse used was so designed and constructed as to exclude, as far as possible, any ingress of insects. The glasshouse was fumigated with nicotine subsequent to seeding and again after emergence of the seedlings.

RESULTS

Seedlings first made their appearance 5 days after irrigation, but the majority emerged 7 days after watering. No symptoms of the disease were

TABLE 1.—*Percentage transmission of squash-mosaic virus through seed collected from infected squash fruits on August 14, 1940, and planted at intervals over a 3-year period*

Age of seeds, in weeks	Total number of plants per sowing	Diseased plants	
		Number	Per cent
Heavy, well-filled seed			
4	425	1	0.23
7	285	0	0.00
11	351	0	0.00
19	1124	3	0.27
23	1091	1	0.09
32	540	2	0.37
35	639	1	0.16
44	873	0	0.00
51	1420	2	0.14
60	1563	2	0.13
69	1559	3	0.19
72	1492	2	0.13
76	1261	3	0.24
83	1202	3	0.25
91	1947	5	0.26
95	1572	3	0.19
98	1503	2	0.13
103	1380	1	0.07
109	1729	2	0.11
112	1294	3	0.23
121	876	0	0.00
130	981	1	0.10
138	735	0	0.00
145	1611	2	0.12
149	1564	3	0.19
156	1477	2	0.13
159	1372	0	0.00
Mean	.....	.....	0.14
Light, poorly filled, deformed seed			
4	228	2	0.88
10	1231	17	1.38
22	970	6	0.62
31	361	8	2.22
50	473	3	0.63
61	1076	13	1.21
74	1370	14	1.02
83	525	4	0.76
90	1209	16	1.32
93	1068	7	0.65
102	959	6	0.62
111	805	4	0.50
120	804	5	0.62
131	1253	14	1.12
140	1623	18	1.11
155	537	4	0.74
Mean	.....	.....	0.96

observed on the cotyledons, though symptoms were usually conspicuously present on the first leaf. Symptoms could be observed immediately upon

the unfolding of the leaves, while they were still quite small. In very few instances were symptoms absent from the first leaf and yet present on the second leaf; rarely did the disease develop on the third leaf and not on the preceding ones.

After the plants had grown for 8 weeks, counts were made to determine the total number of plants and the number diseased. Results of these counts are shown in table 1.

DISCUSSION

The virus causing squash mosaic is definitely seed-transmitted. The relatively low percentage of infected plants arising from infected seed is in harmony with the low amount of the disease usually present in early field plantings. The presence of these infected seedlings provides an excellent and immediate reservoir of the virus, which then can be readily and rapidly spread throughout the planting, either by mechanical means or by insect vectors. This apparently transpires in the field. Fields in which an effort is made to reduce the populations of aphid and *Diabrotica* spp. invariably show less effects of the disease.

Seed stocks should be acquired only from fields free from squash mosaic. The fact that a higher percentage of the disease is transmitted by the light, poorly filled, deformed seed than by the heavy, well-filled seed is of interest and of practical import. If a seed stock is collected from an acreage known to harbor the disease, careful winnowing will remove the majority of irregularly filled seeds and thereby reduce the total amount of the disease that would normally be carried through such seed.

The data given in table 1 show that there was no apparent difference in the percentage transmission in plantings made soon after the seed was harvested and in the final planting, about 3 years later. The virus may be carried by seed at least 3 years old, with no decrease in the percentage of disease transmission.

The fact that a higher percentage of virus is carried by malformed seed than by normal seed, as well as the length of time that the virus remains active, indicates that the latter may be borne internally and not on the surface of the seed coat.

The percentage transmission of squash-mosaic virus through seed of melon and of squash seems very similar. The mean percentage given by Kendrick (8) for muskmelon (0.25 per cent) is of the same magnitude as that determined by the writer for squash (0.14 per cent), for seed of good quality. A higher percentage transmission is prevalent in squash seed of poorer quality (0.96 per cent).

Mahoney (9) probably was dealing with a different virus disease of cucurbits than that discussed herein. The percentage seed transmission that he reports (maximum, 33.3 per cent) is considerably above the values given by Kendrick or the writer (maxima, 2.13 and 2.22 per cent, respectively).

SUMMARY

The symptoms of the squash-mosaic-virus disease are described. The virus is demonstrated to be seed-transmitted. Poor-quality squash seed—that is, light, poorly filled, deformed seed—proved to carry a higher percentage of the causal virus than good-quality, heavy, well-filled seed taken from the same seed population. The virus remains viable in 3-year-old seed, and there is no apparent difference in the percentage of seed transmission of the virus in seed samples sown shortly after harvesting or about 3 years later.

The disease may be controlled to some extent by harvesting squash seed from fields that are free of the disease, and, perhaps, by planting in areas not in close proximity to vector-breeding grounds. If seed is taken from fields known to be infected with squash mosaic, the percentage of seed transmission may be materially reduced by careful winnowing.

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PREVALENCE AND DISTRIBUTION OF STRIPE SMUT OF POA PRATENSIS IN SOME PASTURES OF PENNSYLVANIA[1]

K. W. KREITLOW AND W. M. MYERS[2]

(Accepted for publication January 1, 1944)

Observations by Pammel (6), Clinton (2), Osner (5), Davis (3), and others have emphasized the widespread occurrence of stripe smut of grasses caused by *Ustilago striaeformis* (West.) Niessl. Only limited information is available, however, regarding the prevalence and importance of this smut in Kentucky bluegrass pastures. Allison and Chamberlain (1) reported abundant infection of bluegrass pastures in the vicinity of Richland Center, Wisconsin. Every pasture was infected, and in many cases damage was extensive. Wellhausen, *et al.* (7), examined bluegrass pastures in West Virginia and Pennsylvania and found smut very prevalent. From a total of 504 sod plugs taken at random from different pastures in West Virginia, 15 per cent were diseased. Re-examination of certain pastures revealed an estimated range in smut infection from a trace to about 25 per cent.

Davis (3) found that more than 90 per cent of smutted plants fail to survive periods of drought. Observation has shown that prevalence of smut varies with season of year so that many more diseased plants are noticeable during spring and fall than during hot summer months when droughts generally occur. It has been observed further (4) that bluegrass plants, apparently free of stripe smut when collected, often developed smutted shoots when they were maintained under favorable conditions in a greenhouse. This indicated that a superficial examination of pastures for prevalence of smut in different seasons might be misleading, since some diseased plants could be symptomless at the time of examination, but harbor latent infections of the pathogen that would become manifest under favorable conditions. This suggested that smut infection among plants of *Poa pratensis* may be far more extensive than is generally realized.

MATERIALS AND METHODS

In a preliminary survey of more than 75 bluegrass pastures in Pennsylvania, the writers found very few that appeared to be free of stripe smut, while many bore extensive infection.

During the fall of 1942, each of 13 representative pastures in the vicinity of State College, Pennsylvania, was divided by estimation into 4 sections, and approximately 50 sod plugs, 1.5 inches in diameter, were collected at random from each section. The sod plugs were examined for smut on the day following collection, and the number of plugs containing smutted plants was recorded.

[1] Contribution No. 59, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the Northeastern States.

[2] Associate Pathologist and Geneticist, respectively.

In order to preserve the plugs for further examination, those from each section of a pasture were placed in rows in a flat containing wet peat moss. Thus, each plug was kept separate and the plants therein were maintained under favorable growing conditions. Each of the 2600 plugs was examined for smut at 1-, 2-, 3-, and 5-month intervals. The plants in each plug were clipped periodically to maintain them at a height convenient for examination.

RESULTS

Smutted plants (Table 1) were obtained from each of the 13 pastures. At the time of sampling, the proportion of plugs with smutted plants varied for different pastures from 0.5 to 11.4 per cent. When the plugs were re-examined one month later, nearly all samples showed an increase in number of smutted plugs. The collections from some pastures contained more than twice as many smutted plugs as were detected at the first examination.

TABLE 1.—*Per cent of plugs with smut-infected plants at different periods of examination and total per cent smut*

Pasture No.	Months after collection					Total[a]
	0	1	2	3	5	
70	7.6	12.0	18.1	19.5	17.7	30.0
71	4.9	9.3	14.2	13.0	11.7	25.9
72	4.0	4.0	5.0	6.5	5.5	10.0
73	0.5	1.5	2.1	2.0	1.5	4.5
74	5.5	6.0	9.0	11.0	10.0	16.5
75	11.4	15.3	18.1	18.7	14.9	31.0
76	3.9	9.9	20.8	25.3	25.2	34.4
77	5.5	8.4	13.4	9.9	14.9	21.8
78	11.1	10.5	13.5	17.0	13.0	23.0
79	10.6	12.6	15.2	14.2	12.7	23.9
80	9.0	16.3	18.2	17.2	19.1	29.5
81	3.9	7.7	7.7	7.8	8.3	14.6
82	2.0	5.0	5.0	4.5	4.6	11.0

[a] Total per cent smutted plugs recorded during the entire observation period.

There was no evidence that increase in number of smutted plugs from one examination period to the next was due to spread of infection among plugs in the flats. This was demonstrated by plotting the location of smutted plugs after each examination. If transmission of smut were taking place, one would expect the healthy plants in plugs adjacent to diseased plants to be the first to show symptoms of infection. However, the location of additional plugs containing smutted plants appeared to be random.

When the plugs were examined 2 and 3 months later, a further increase in the number of smutted plugs was found in some collections. This indicated that most of the smut present among random samples from different pastures was detected by growing the plugs for at least 3 months under favorable conditions in the greenhouse. At the last examination, the collections from most pastures showed a decrease in number of smutted plugs;

however, 1.5 to 25.2 per cent of plugs from the different pastures still contained smutted plants.

The decrease in smut observed in some collections was due to death of the diseased plant or plant part in some of the plugs. Since only healthy plants or at least plants showing no symptoms of disease were left, classification of such plugs was changed from diseased to healthy.

A measure of total smut present among collections of plugs from each pasture was determined by adding together all plugs recorded as smutted during the 5-month observation period. This figure included plugs in which smutted plants succumbed or those that contained plants that lost their smut symptoms, as well as smutted plants in plugs that were designated as healthy at earlier examinations. Since a record was kept regarding the status of

TABLE 2.—*Distribution in per cent of smutted plugs within different parts of four representative pastures*

Pasture No.	Section	Months after collection				
		0	1	2	3	5
72	1	4.0	4.0	4.0	6.0	9.0
	2	12.0	10.0	14.0	20.0	14.0
	3	0.0	0.0	0.0	0.0	0.0
	4	0.0	2.0	2.0	0.0	0.0
73	1	0.0	1.9	0.0	3.8	1.9
	2	2.1	2.1	8.3	4.2	2.1
	3	0.0	0.0	0.0	0.0	0.0
	4	0.0	2.0	0.0	0.0	2.0
76	1	3.8	5.8	13.5	17.3	25.0
	2	3.9	9.8	19.6	31.4	23.5
	3	0.0	8.2	18.4	22.4	22.4
	4	8.0	16.0	32.0	30.0	30.0
80	1	8.2	10.2	12.2	20.4	16.3
	2	0.0	4.0	8.0	6.0	4.0
	3	3.8	18.9	26.4	26.4	30.2
	4	24.0	32.0	26.0	16.0	26.0

each plug at each examination, any plug that had displayed a smutted plant at any time during the observation period was recorded as smutted. The data so recorded are shown in the last column of table 1 and illustrate the extensive smut infection present in the pastures sampled. The least proportion of smutted plugs recorded was 4.5 per cent from pasture No. 73. The greatest proportion of smutted plugs collected, 34.4 per cent, came from pasture No. 76. Of the 13 pastures sampled, 8 yielded more than 20 per cent smutted plugs.

DISTRIBUTION OF STRIPE SMUT IN SOME PASTURES SAMPLED

The distribution of stripe smut in pastures was studied by recording separately the number of smutted plugs collected from the 4 parts of each pasture. The data from table 2 show that some pastures varied appreciably in the amount of smut present in different areas. For example, sections 3 and 4 of pasture No. 72 showed no smut at the time they were sampled, while

sections 1 and 2 of the same pasture contained smut to the extent of 4.0 and 12.0 per cent, respectively. The number of smutted plugs in sections 1 and 2 increased eventually to a maximum of 9.0 and 20.0 per cent. Similarly, there were wide fluctuations in different parts of pasture No. 80. At the first examination, the number of smutted plugs from different sections of this pasture varied from 0 to 24.0 per cent. At the last examination, the variation was from 4.0 to 30.2 per cent.

The results from pastures 73 and 76 (Table 2) are illustrative of those obtained from pastures bearing a fairly uniform distribution of smut. Despite some fluctuation in number of smutted plugs at different examinations, the final examination revealed a rather uniform amount of smut among the samples collected from these pastures.

DISCUSSION

From the data presented, one may conclude that prevalence of stripe smut of *Poa pratensis* in some pastures of Pennsylvania is much greater than superficial examinations would ordinarily disclose. It is well known that the amount of smut present during different seasons fluctuates, depending on weather conditions. Under favorable growing conditions of spring and fall, considerable numbers of smutted plants are noticeable. That the amount of smut visible in a bluegrass pasture at any one time is not necessarily representative of the total amount present was demonstrated by the marked increase in number of sod plugs containing smutted plants when the samples collected from different pastures were grown under favorable conditions in a greenhouse. The increased number of smutted plugs was apparently due to expression of symptoms among plants that were symptomless at the time they were collected and to dormant infections among some plants.

The high percentage of smutted plugs collected from some of the bluegrass pastures leaves little doubt that stripe smut may be important in reducing yields. The observed decrease in prevalence of smut during hot summer months is probably due to death at least of the above-ground parts of many diseased plants. This may account, to some extent, for the so-called "drying-up" of bluegrass pastures during the summer season. With the onset of unfavorable environmental conditions for the host, stripe smut might weaken plants to a point where they would succumb to the infection or be predisposed to invasion by other pathogens.

The pastures studied in these investigations varied in size from a few to many acres. Within some of the pastures there was a wide range in topography, soil type, fertility, and density of Kentucky bluegrass. Within the limits of the sampling in this investigation, there was no apparent consistent relationship between these factors and variations in prevalence of smut infection. It is possible that more extensive investigations would reveal such relationships. Other factors that may have influenced variations within pastures are age of pasture, distribution of susceptible strains of Kentucky

bluegrass, and management practices and other conditions affecting the spread of the pathogen and survival of diseased plants. These factors also may have been important in conditioning differences between pastures.

The results of these investigations emphasize the importance of a further study of the life history, epidemiology, ecology and genetics of the pathogen causing stripe smut.

SUMMARY

Examination of *Poa pratensis* plants in sod plugs collected from different parts of each of 13 representative pastures in Pennsylvania revealed that stripe smut caused by *Ustilago striaeformis* was present at the time of sampling to the extent of 0.5 to 11.4 per cent.

The sod plugs from each pasture were maintained separately and observed periodically. In most cases, there was an increase in number of plugs containing smutted plants. This was attributed to expression of symptoms among plants that were symptomless at the time they were collected and to dormant infection.

The total number of plugs recorded as containing smutted plants during a 5-month observation period varied for different pastures from 4.5 to 34.4 per cent.

Observations on distribution of stripe smut in different parts of pastures revealed that some contained a fairly uniform infection over the entire pasture while others contained variable amounts for different areas.

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CARROT BACTERIAL BLIGHT AS IT AFFECTS THE ROOTS

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(Accepted for publication January 3, 1944)

A scab disease of carrot roots, serious in California (1) has been found to be caused by the bacterial blight organism, *Phytomonas carotae* (Kendrick) Bergey *et al.*, 1939, described by Kendrick (2) in 1934 as the cause of a disease of the leaves and umbels. The root phase of this disease is probably identical with a scab reported as an important market blemish in California-grown carrots in 1936 by Ramsey (3, p. 86) and Ramsey and Wiant (4, p. 43, and Pl. 13–B). It was first observed in California in 1937 in the Santa Maria Valley, and is prevalent near Soledad in the Salinas Valley in irrigated fields of sandy soil, cropped repeatedly to carrots.

The bacterial-blight organism has been isolated from the root lesions, and its pathogenicity proved by atomizer inoculation of carrot foliage. Root infection was obtained with this organism in carrot plants grown from sterilized seed in flats of sterilized soil to which a suspension of the organism had been added.

SYMPTOMS

Infection may occur at any point on the surface of the root (Fig. 1, A, D). Late infection of fairly large roots causes rather shallow, scabby lesions, often very numerous (Fig. 1, A, B, C, D). Young lesions are very small, brown or maroon spots, which may develop into slightly raised pustules or slightly sunken, laterally elongated craters with flakes of black necrotic tissue at the center (Fig. 1, A, B, Fig. 2, B). These lesions often bear gray masses of bacterial ooze. Examination of washed roots with a hand lens often reveals another type of incipient lesion associated with this disease, a shallow, water-soaked or greasy fleck 2 or 3 mm. in diameter, involving only the epidermis (Fig. 2, B). Such lesions may develop a brown necrotic spot in the center.

Larger lesions are elongated laterally (Fig. 1, A, B, C, Fig. 2, A, C). The necrotic tissues in the lesion crack open and a copious bacterial exudate embedding soil particles and fragments of the black necrotic tissue is largely responsible for the raised, black, scabby character of the lesion. When the roots are washed the lesions emit fresh gray masses of bacterial exudate. Kendrick (2) pointed out this tendency of the blight lesions on the umbels to ooze copiously.

Because of its interference with normal root enlargement, early infection may result in a sunken area with a scab (Fig. 2, F) or blackened canker (Fig. 2, D) at the center or a healed-over pocket of blackened necrotic tissue (Fig. 2, E, F). These internal or buried lesions in fairly normal appearing roots are particularly objectionable because such roots cannot be culled out in the field or packing sheds. Occasionally large, rough, sunken cankers

are formed by coalescence of early lesions (Fig. 2, D). Early infection may be fairly deep-seated, and, although usually arrested and overgrown, may be fatal to young plants.

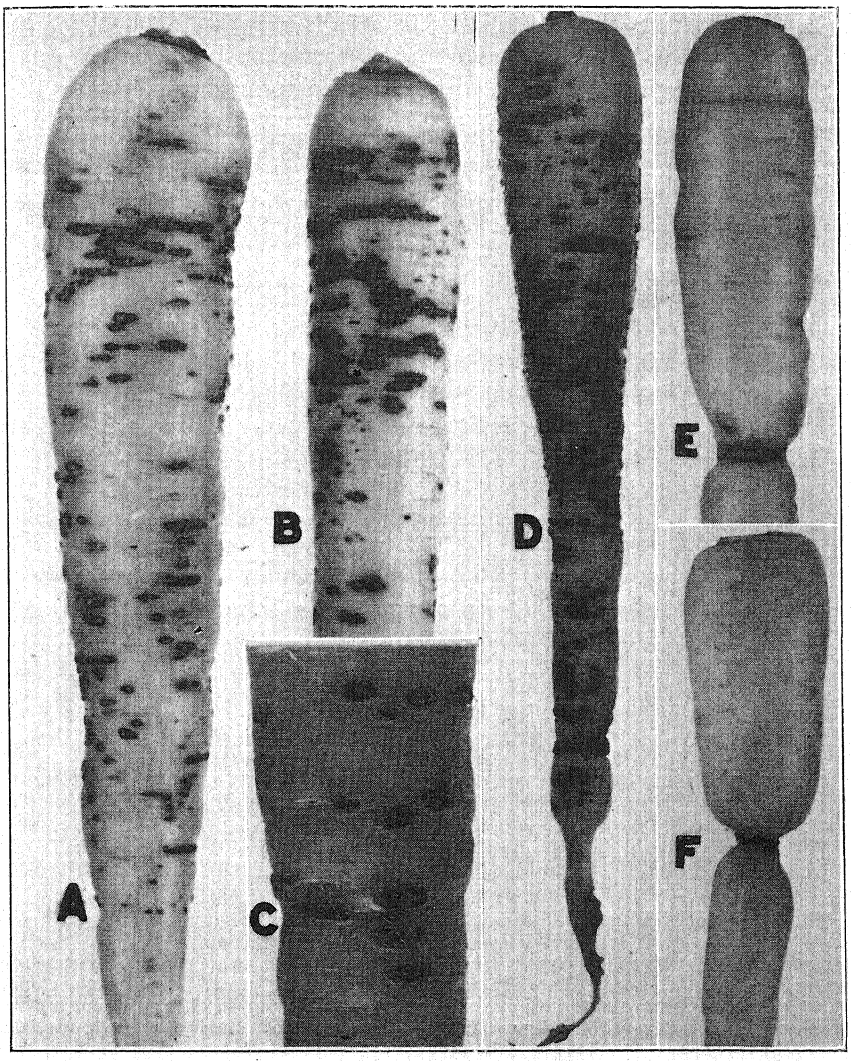


FIG. 1. Root symptoms of carrot bacterial blight. A to D. Raised scabby lesions, elongated laterally. In B and D some very small lesions are shown. E and F. Encircling lesions and root constriction resulting from early infection.

Frequently early infection results in a sharply sunken encircling constriction of the root, which may cause it to break in two when pulled (Fig. 1, E, F). Secondary infection of blight lesions by rot-producing fungi is of frequent occurrence.

Internal infection of the crown of old roots used for seed production has

been found in the form of discolored strands (Fig. 2, G) proceeding downward, presumably from previously infected leaves.

PERSISTENCE OF ORGANISM IN THE SOIL

In the Soledad region where root infection is prevalent, carrots for eastern shipment have been grown more or less continuously and at all seasons of the year, some fields having a history of 4 carrot crops in 3 years. Soil

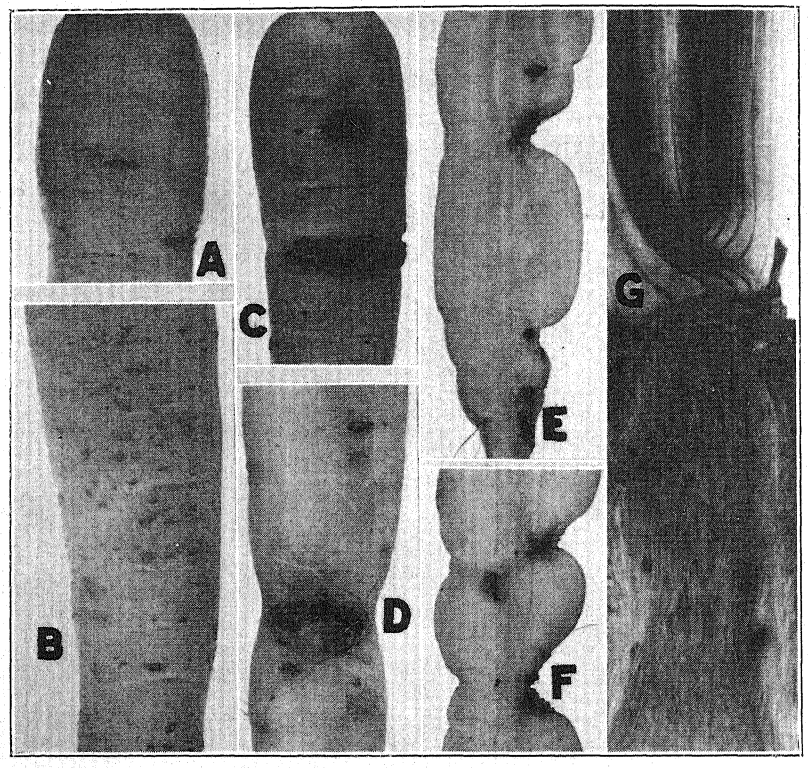


FIG. 2. Root symptoms of carrot bacterial blight. A. Laterally elongated scab lesions and very small incipient lesions. B. Two types of incipient lesions, laterally elongated, sharply delimited, black specks and freckle-like, skin-deep, watersoaked or greasy blotches. C. Large, laterally elongated, encircling scab. D. Sunken canker, resulting perhaps from coalescence of early infections. E and F. Longitudinal sections through root showing constrictions and internal pockets of black necrotic tissue resulting from early infection. G. Longitudinal section of crown of old seed root showing dark strands of necrotic infected tissue that extend downward from infected leaf bases.

conditions are evidently favorable for root infection throughout most of the year, since none of the crops representing different dates of planting seem to escape. On the other hand, foliage infection in these fields has been observed only in the spring on crops exposed to the winter rains and is believed to have little or no current relation to root infection.

Kendrick (2) has shown that foliage infection may come from the soil. That the soil in the Soledad carrot district is heavily contaminated with the

blight bacteria has been proved by the abundant root infection obtained on carrots grown from hot-water-treated seed in flats of soil brought in from diseased fields to the greenhouse. In fact, infection was obtained after the soil had been in dry storage for 12 months.

The copious oozing of the bacteria from the root lesions and the dangerous harvest practice of leaving the culls in the field to be plowed under provide for abundant soil infestation.

The disease is much worse in fields repeatedly cropped to carrots. For example, counts made at Soledad in January, 1943, showed conspicuous infection on 12 per cent of the roots in new soil as compared with 50 per cent in old carrot soil. These plots were planted with sterilized seed, and the presence of the organism in the soil not previously in carrots may possibly be explained by the deposits of wind-blown, contaminated soil from old carrot fields nearby. A very strong afternoon wind that drifts the soil is a daily phenomenon in that district at certain seasons.

SEED DISSEMINATION AND SEED DISINFECTION

Kendrick (2) showed that bacterial blight occurred abundantly in the umbels of carrots grown for seed and that artificially contaminated seed gave rise to infected seedlings. Seed from infected and healthy umbels was collected in the Delta region in June, 1940. Seed from infected umbels was planted in flats of sterilized soil in the greenhouse in late October and among about a thousand seedlings examined, 4.3 per cent showed foliage infection. In field plots planted with this seed in July, 1940, the plants were grown until they bore seed. In September, 1941, foliage and umbel infection was found on 8 of the 199 plants grown from seed collected from diseased umbels, whereas no disease developed among the 236 plants grown from seed collected from healthy umbels.

The same carrot seed from infected umbels was used to test various methods of seed disinfection. In greenhouse tests in sterilized soil, started in January, 1941, 9 per cent of the several hundred plants grown from untreated seed were infected, whereas no infection occurred among a similar number of plants grown from seed treated in hot water, 52° C., for 10 minutes. Field plots were planted in Berkeley in the spring of 1941 in soil not previously in carrots, and disease counts were made in May. In the check plot planted with seed dipped in water, 47 per cent of the seedlings were infected, whereas no infection occurred in the plots planted with seed treated for 10 minutes in hot water at 52° C. or for 10 minutes in a 1:1000 solution of mercuric chloride. In the field test previously mentioned in which the seed was planted in July, 1940, and the records taken over a year later, 4 per cent of the plants from unsterilized seed were infected, 3 per cent, where seed treated in a 1:1000 solution of mercuric chloride was used, and none where the seed treatment was hot water, 52° C., for 10 minutes.

Large field plots have been planted with hot-water-treated commercial seed at various times during 1942 and 1943, in the Soledad district, with no

indication of any injury from the treatment; but the presence of general soil infestation with the blight bacteria rendered the seed treatment ineffective against the disease.

SUMMARY

Black, scabby lesions on carrot roots are caused by the carrot bacterial blight organism, *Phytomonas carotae.* Deep constrictions and internal pockets of necrotic tissue may result from early infection.

The organism is harbored in the soil and the disease is serious in fields cropped repeatedly to carrots.

Hot-water treatment of the seed is effective against seed-borne infection.

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THE POTENTIAL IMPORTANCE OF RACE 8 OF PUCCINIA GRAMINIS AVENAE IN THE UNITED STATES[1]

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(Accepted for publication December 31, 1943)

Race 8 of *Puccinia graminis avenae* was sufficiently prevalent in 1943 to cause concern regarding its possible future effect on the performance of Vicland, Boone, Tama, and several other recently developed oat varieties, mostly derived from Victoria × Richland crosses. Because of their yielding ability and resistance to stem rust, crown rust, and smuts, these new varieties have been rapidly replacing older varieties of oats. They owe their stem-rust resistance to the Richland parent, one of the differential varieties used in identifying races of *P. graminis avenae.*

Although Richland is susceptible to 7 of the 13 races of *Puccinia graminis avenae* now known, none of them has hitherto been sufficiently prevalent in the United States to be of practical importance, and only 2 races (8 and 10) have been found commonly. Richland and the varieties that derived their resistance from it are not susceptible to races 2 and 5, the only races prevalent enough to be of practical importance during the past 15 or 20 years (3, 5, 6, 7, 8). Nevertheless, as pointed out by the writers and Cotter (6, 8), there have been indications recently that races 8 and 10 might increase sufficiently in prevalence to endanger those varieties with the Richland type of resistance.

The prevalence of races, expressed as percentages of total isolates for 1939 to 1943, inclusive, is shown in table 1.

Only 6 races were represented in more than 1,200 isolates during the past 5 years; and, for practical purposes, the number may be considered as 4, since races 2 and 5 may be combined; likewise 8 and 10. Races 2 and 5 differ only in their effect on the variety Jostrain, race 2 producing type 4 and race 5 producing type X. There is a similar difference between races 8 and 10 on Jostrain. When the combinations are made, it is apparent that races 2 and 5 comprised most of the rust during the past 5 years, races 7 and 12 were almost negligible, and races 8 and 10 were present in small but appreciable amounts in 1940 and 1941 and in considerable amounts in 1943

[1] Cooperative investigations between the United States Department of Agriculture and the Minnesota Agricultural Experiment Station. Paper No. 2114 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

Many of the collections of rusted oats were made by members of the barberry eradication organizations in various States. The following also contributed a number of valuable collections: D. G. Fletcher, of the Rust Prevention Association, Minneapolis, Minn.; I. M. Atkins, E. S. McFadden, H. C. Murphy, R. G. Shands, and T. R. Stanton, of the Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture; and T. E. Stoa, of the North Dakota Agricultural Experiment Station.

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(Table 1). Of the races isolated during the 5 years, only 8 and 10 attack Richland and its derivatives heavily (3).

In 1943 race 8 caused such conspicuous rust on the new rust-resistant varieties derived from Richland × Victoria crosses as to evoke independent comment from several plant pathologists, plant breeders, and men engaged in rust surveys. Although the severity of infection on the resistant varieties was relatively low in most cases, the infection type indicated complete susceptibility. That the rust actually was caused by race 8 is clear, as isolates of this race were obtained from Vicland, Boone, Tama, Control, from an unnamed line of Richland parentage, and from fields of unknown varieties. Race 8 always was isolated in pure form from the resistant varieties, but race 2 tended to predominate when both were isolated from collections of nonresistant varieties. In each of 5 such collections, the ratios between races 2 and 8 were 50:50, 85:15, 85:15, 80:20, and 50:50. These ratios are determined by estimating the number of type–1 and type–4 pustules on

TABLE 1.—*The prevalence of races of Puccinia graminis avenae in the United States from 1939 to 1943, inclusive*

Year	Race and percentage of total isolates								Total number of isolates
	2	5	7	8	10	12	2+5	8+10	
1939	55.7	41.6	1.2	1.2	0.3	0.3	97.3	1.5	251
1940	53.1	39.9	.....	2.4	3.8	0.7	93.0	6.2	286
1941	55.7	38.4	.....	1.6	3.3	1.0	94.1	4.9	305
1942	65.5	32.4	.....	0.4	1.7	.....	97.9	2.1	232
1943[a]	79.1	.....	.....	20.2	.....	0.7	79.1	20.2	421

[a] No distinction is made between races 2 and 5, nor between 8 and 10, in 1943.

Richland, produced by races 2 and 8, respectively, when differential varieties are inoculated in the greenhouse.

Race 8, originally found in Canada (2, 4), was collected in the United States for the first time in 1937, in Iowa, Wisconsin, and Pennsylvania. In 1938 it was found only in Virginia. During the first two years, therefore, it was found only in barberry-infested areas; and in 1939 it appeared in Ohio, Wisconsin, and Missouri. In 1940 it was found in Kansas and Texas, as well as in Iowa, Wisconsin, and Minnesota. It appears, therefore, to have become established first in the north and northeast, where barberries become rusted, and then to have extended southward and westward. Race 10 was first identified by Cotter (1) from rusted oats growing near barberries in Jefferson County, Wisconsin, under circumstances that make it virtually certain that the rust originated on the bushes. It appears, therefore, that both races 8 and 10 began their careers near barberries.

Race 8 then spread southward into Texas, where the uredial stage appears to have survived the winter of 1942–43; and the rust spread northward during the growing season of 1943. This sequence of events seems clear, although the evidence necessarily is circumstantial. Race 8 was isolated from rusted oats in Texas in the early spring of 1943 and was isolated subse-

quently from collections made in Colorado, Iowa, Kansas, Michigan, Minnesota, Missouri, Montana, Nebraska, North Dakota, Oklahoma, Pennsylvania, South Dakota, and Wisconsin (Fig. 1). Although inoculum of race 8 came from the South, it probably would not have been there except for barberries in the North. The fact that new or rare races have two possibilities of surviving the winter—uredial overwintering in the South and the telial-aecial sequence in the North and East—makes it doubly desirable to impede their possible production in the sexual stage on the barberry. When once produced, there is a possibility that they may perpetuate themselves in the uredial stage by traveling *via* air currents from north to south in the fall and from south to north in the spring and summer.

Despite their low prevalence, races 8 and 10 have been rather widely distributed. Even prior to 1943, race 8 was found in the following States during the period 1939–1942, inclusive: Iowa, Kansas, Minnesota, Missouri,

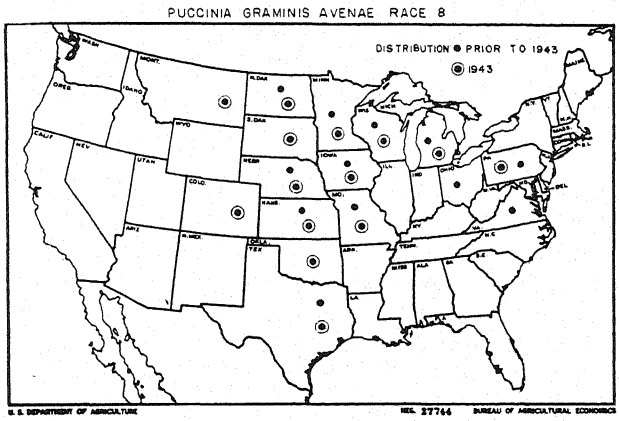


FIG. 1. Distribution of *Puccinia graminis avenae* race 8 in the United States.

Nebraska, North Dakota, South Dakota,[4] Ohio, Pennsylvania, Texas, and Wisconsin. During the same period, race 10 was found in Texas, Nebraska, Iowa, North Dakota, Minnesota, Wisconsin, Michigan, Virginia, and Pennsylvania.

There can be no certainty regarding the future of race 8 or other races to which Richland derivatives are susceptible. It is clear, however, that both 8 and 10 are rather widely distributed geographically and that there was considerable inoculum of race 8 during the summer of 1943. Given conditions favorable for extensive overwintering of the uredial stage in the South or for abundant development on barberry bushes in the North, or both, followed by favorable conditions for rust development during one or more growing seasons, this race might soon become strongly established. On the other hand, any one of several unfavorable circumstances might result in its subsidence. The tendency for the new rust-resistant oat varieties to reduce the prevalence of races 2 and 5 may favor the increase of race 8 and other similar races by eliminating competition. It is clear that there have

[4] Not included in figure 1.

been decided and important changes in prevalence of races of *Puccinia graminis tritici* (5, 6, 7, 8); and equally great changes may or may not occur in *P. graminis avenae*.

White Tartar, another of the differentials used in identifying races of *Puccinia graminis avenae* and also used as a stem-rust-resistant parent in crosses, is very resistant to races 2, 5, 8, and 10, but is susceptible to races 3, 4, 6, 7, 12, and 13. During the past 20 years these races either have not been found in the United States or have been so rare as to be of no consequence; however, during the past 5 years, 1939 to 1943, inclusive, race 7 was isolated from collections made in 1939 in barberry-infested areas in Wisconsin and Pennsylvania. It also was obtained from Oklahoma. In 1939 race 12 was isolated from aecial material collected in Pennsylvania, in 1940 it was found near barberries in the same State, in 1941 it was isolated 3 times from aecia from Pennsylvania, and in 1943 it was isolated from barberry areas in Pennsylvania and New York. The other races that attack White Tartar were not isolated in the United States, although races 1 to 10, inclusive, were found in Canada during the period 1925–1936, inclusive, according to Gordon and Welsh (2) and Margaret Newton (4).

SUMMARY AND CONCLUSIONS

The primary object of this paper is to call attention to the increase in prevalence in 1943 of race 8 of *Puccinia graminis avenae*. This increase may be only temporary or it may be relatively permanent, depending on conditions affecting the various phases of rust development in the near future.

The increase of race 8 in 1943 is important because it is evident that this race and race 10, which is combined with it for practical purposes, can cause heavy infection on Richland oats and on such varieties as Vicland, Boone, Tama, and others, mostly derived from Victoria × Richland crosses, and having the Richland type of resistance. Not only seedlings but also adult plants of these varieties are susceptible, confirming conclusions of Levine and Smith (3) regarding agreement between reaction of seedlings and adult plants of oats to physiologic races of *Puccinia graminis avenae*. As race 8 was isolated a number of times, especially in 1943, from large uredia on the hitherto generally resistant varieties, and as there was abundant rust on them in some localities, there is no question regarding their susceptibility. The real question is whether race 8 and the very closely related race 10 will increase, as certain races of *P. graminis tritici*, notably races 56 and 17, increased in recent years. Races 8 and 10 have been rather widely distributed geographically in the United States for several years, and the possibility of an increase in prevalence must, therefore, be recognized. Although a further increase in races 8 and 10 might jeopardize the varieties that derived their resistance from Richland, there are no present indications of an increase in the United States of those races to which White Tartar and its resistant derivatives are susceptible. Moreover, there are

indications from the work of Welsh (9) that it may be possible to obtain varieties combining resistance to all or most of the races now known.

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THE ANTAGONISM OF SOIL ORGANISMS TO FUSARIUM OXYSPORUM CUBENSE[1]

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(Accepted for publication October 6, 1943)

In the banana-producing sections of Jamaica it is common knowledge that some areas have a natural resistance to Panama disease, while other, near-by areas lack this resistance. Wardlaw (7) has pointed out that there are many unexplained aspects of the Panama-disease problem and refers to a situation on the lower Ullua River in the Republic of Honduras, where, notwithstanding periodic inundations by flood waters carrying débris from affected areas higher up, large areas have remained in a high state of production for a period of about 50 years.

An explanation of this natural resistance is suggested by experiments conducted by Alexopoulos, Arnett, and McIntosh (2) in 1938, which gave evidence of antibiosis between bacteria and fungi. Continuing this work Alexopoulos (1) tested out the ability of 80 Actinomyces species to inhibit the growth of *Colletotrichum gloeosporioides* on maltose agar. They were classified as 35 non-inhibitors, 31 weak inhibitors, and 14 strong inhibitors. Alexopoulos and Herrick (3) point out that actinomycetes differ in their ability to inhibit the growth of a given fungus in culture under the conditions of the experiment. Waksman, Horning, Welsch, and Woodruff (6) found that actinomycetes possessing antagonistic properties against bacteria and fungi were widely distributed in nature, especially in soils and composts. Two hundred and forty-four cultures were isolated at random from different soils; of these, 106 cultures possessed some antagonistic properties, and 49 were highly antagonistic.

An investigation into the character of this natural resistance of soils to Panama disease was, therefore, undertaken, resulting in the isolation of certain promising soil organisms (4). Most of the soil organisms encountered in this work have been actinomycetes (5), but careful taxonomic work has been deferred. These organisms fell into 3 classes: those (a) that apparently increased the growth of the Panama-disease fungus, (b) that did not interfere with the growth, and (c) that were antagonistic to the *Fusarium*.

MATERIALS AND METHODS

Soil samples were collected from various parishes in Jamaica, an effort being made to get soils of different composition and texture. Sixty-six samples were used that had been collected from St. Mary (45), St. James (13), St. Andrew (4), and St. Thomas (4).

A soil solution was made by shaking 20 g. of soil with water up to 1 liter. The mixture was filtered after about 30 minutes. This solution was used

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support of this research.

with Parke, Davis and Co. granular agar for 2 per cent agar media. Each isolation and each test was made on the soil solution from which the soil organism was taken. The isolations were made by dilution methods with 0.00001 to 0.000001 g. of soil per plate.

The first tests were conducted in plates by making a line of the soil organism across the plate and introducing *Fusarium oxysporum cubense* about one-half inch from the soil culture. Later, a long, thin slant was made in test tubes and the same procedure followed. The soil organism was introduced by making a cut across the agar with a flattened needle, curved at the end, while the *Fusarium* was introduced about one-half inch above. Measurements of the mycelial growth of the *Fusarium* were made and recorded by the use of a low-powered microscope.

RESULTS

The soil organisms that exhibited antagonism to the Panama-disease fungus under the conditions of the experiment were placed in 3 classes:

1. Slightly antagonistic; organisms that retard the growth of *Fusarium oxysporum cubense.*
2. Antagonistic; organisms that have the ability to produce an area free from mycelium of *Fusarium oxysporum cubense.*
3. Very antagonistic; organisms that have pronounced ability to dissolve or inhibit the growth of *Fusarium oxysporum cubense.*

The isolations under observation from the 66 soil samples collected numbered 7642; 1020 of these were grown with the Panama-disease fungus. The antagonistic organisms found were classified as follows: (1) slightly antagonistic, 66; (2) antagonistic, 39; and (3) very antagonistic, 17 (Table 1). Only 15 of the soil samples produced very antagonistic organisms. There were 122 antagonistic organisms in all taken from the 66 soil samples; 17 samples produced no antagonistic organisms, 19 produced 1, 11 produced 2, 9 produced 3, 4 produced 4, 2 produced 5, 3 produced 6, and 1 produced 10. The antagonistic organisms were not evenly distributed, as was shown by the fact that 10 of the soil samples produced 54 antagonistic cultures, while 56 produced only 68.

A further experiment was conducted on Newry soil-solution agar to test out the ability of these antagonistic organisms to affect the growth of the Panama-disease fungus on soils other than the one from which they were taken. The 17 actinomycetes used were classified as very antagonistic (5), antagonistic (11), and slightly antagonistic (1). Eight of these organisms were antagonistic to *Fusarium oxysporum cubense* on the Newry soil-solution agar, while 9 did not exhibit the antagonism that they had shown on their own soil-solution agar. Of the 8 actinomycetes antagonistic to the *Fusarium* on Newry soil-solution agar 2 were listed as very antagonistic and 6 were listed as antagonistic on their own soil-solution agar.

From experiments conducted up to the present time it appears that the antagonism of actinomycetes varies with the soil solution, but the growth

TABLE 1.—*The effect of soil organisms on Fusarium oxysporum cubense growing on soil-solution agar*

Soil sample	Isolates	Number tested	Slightly antagonistic	Antagonistic	Very antagonistic
St. Mary					
1. Frontier	124	5	.....	.....	1
2. "	150	5	.....	.....	.....
3. "	182	5	.....	.....	.....
4. "	85	4	.....	.....	.....
5. "	87	4	.....	.....	.....
6. "	15	15	.....	.....	.....
7. "	29	29	1	.....	1
8. "	18	18	1	.....	.....
9. "	22	22	2	.....	.....
10. "	24	24	1	1	.....
11. "	15	9	.....	3	1
12. Gray's Inn	26	26	3	.....	1
13. " "	669	24	2	.....	.....
14. " "	550	21	1	.....	.....
15. " "	580	20	3	.....	.....
16. " "	27	23	4	6	.....
17. " "	114	17	1	1	.....
18. " "	326	23	1	1	.....
19. Newry	12	12	.....	.....	.....
20. "	266	20	.....	.....	1
21. "	38	15	3	.....	.....
22. "	25	10	.....	.....	2
23. Albion	15	15	.....	.....	.....
24. Clermont	15	15	.....	.....	1
25. Salem Manse	18	18	2	.....	.....
26. " "	160	20	.....	.....	.....
27. " "	54	20	2	.....	1
28. " "	81	20	1	.....	.....
29. Rosend	25	10	.....	.....	.....
30. "	43	15	.....	.....	1
31. "	69	10	.....	.....	1
32. Green Castle	65	20	.....	.....	.....
33. " "	127	20	.....	.....	.....
34. " "	14	10	1	.....	.....
35. Cape Clear	141	10	1	1	1
36. " "	75	20	5	.....	.....
37. " "	512	20	.....	.....	.....
38. " "	124	20	1	.....	.....
39. " "	55	10	2	1	.....
40. " "	618	20	1	.....	.....
41. " "	39	10	1	1	1
42. " "	282	20	.....	.....	.....
43. " "	107	10	1	.....	.....
44. " "	143	10	.....	.....	.....
45. Harmony Hall	161	16	1	.....	.....
St. James					
46. Guilsboro	5	5	.....	.....	.....
47. "	8	5	1	.....	.....
48. "	75	20	2	1	.....
49. "	142	16	5	1	.....
50. "	118	18	.....	1	.....
51. "	16	10	1	.....	.....
52. "	41	12	.....	.....	.....
53. "	7	7	2	2	.....
54. "	26	12	.....	.....	.....
55. "	136	20	.....	1	.....

TABLE 1.—(*Continued*)

Soil sample	Isolates	Number tested	Slightly antagonistic	Antagonistic	Very antagonistic
56. Guilsboro	111	15	3	2	.....
57. "	81	18	.....	4	2
58. Ducketts	46	20	1	1	.....
St. Thomas					
59. Amity Hall	64	20	4	2	.....
60. Holland	47	12	.....	1	.....
61. "	69	12	.....	1	1
62. "	74	12	1	.....	.....
St. Andrew					
63. Kirklands	27	22	3	.....	.....
64. "	44	12	.....	2	1
65. "	89	20	1	1	.....
66. "	89	22	.....	4	.....
Totals	7642	1020	66	39	17

of the Panama-disease fungus tends to be at about the same rate on all the soil solutions examined.

SUMMARY

Of the soil organisms, mostly actinomycetes, isolated from 66 soil samples in Jamaica, 122 exhibited antagonism to *Fusarium oxysporum cubense*. They were classified as 66 slightly antagonistic, 39 antagonistic, and 17 very antagonistic.

The antagonistic organisms were not evenly distributed in the soil samples, as 10 of the 66 samples had 44.2 per cent of the antagonistic organisms.

Actinomycetes antagonistic to *Fusarium oxysporum cubense* in their own soil-solution agar were not always antagonistic when tested in other soil-solution agar.

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XANTHOMONAS VIGNICOLA SP. NOV. PATHOGENIC ON COWPEAS AND BEANS

WALTER H. BURKHOLDER

(Accepted for publication January 1, 1944)

In August, 1942, A. A. Dunlap sent the writer specimens of cowpea plants (*Vigna sinensis* Endl.) showing large fissures and cankers on the stems, and later,[1] he published a description of the disease as it appears in the field under Texas conditions. The writer, without having seen naturally infected plants in the growing condition, made careful examinations of the stem lesions of these specimens and found that a great many bacteria were present in the necrotic tissue that evidently were the cause of the disease. Dilution plates from a number of the cankers gave rise to yellow bacterial colonies similar in growth characteristics to those of *Xanthomonas phaseoli* (E. F. Smith) Dowson. A search through literature, however, showed no report of this pathogen on the cowpea, and past experiences of the writer had shown that all attempts to infect this crop with the bean blight organism met with failure. No cowpea plants were available at the time of isolation with which to test the pathogenicity of these bacteria, but there were a number of young Red Kidney beans growing in the greenhouse. Inoculations on these plants, made by introducing the bacteria into a wounded stem, demonstrated that the organism was extremely virulent to this crop, and in approximately 3 weeks the plants were dead.

These results led to an investigation to determine the relationship of the cowpea pathogen to *Xanthomonas phaseoli,* and the ability of the two to infect beans, cowpeas, and related plants. The diseased cowpeas received from Texas were of the Chinese Red variety, so this variety was used in the experiments. Seed was kindly furnished by Dr. Dunlap. The bean variety used was the Red Kidney that is very susceptible to *X. phaseoli* and that had been shown to be very susceptible to the cowpea organism. In the inoculation experiments, 6 isolates of the cowpea pathogen, and 2 isolates of *X. phaseoli* were employed. The latter (XP4 and XP14) were 1 and 2 years old but still very pathogenic on beans. These 8 isolates proved pathogenic on the Red Kidney plants with approximately equal virulence. The symptoms were those always associated with *X. phaseoli.* It appeared in these experiments that the cowpea pathogen, after being in culture for 4 months, had lost its extreme virulence for the bean or that, during the second set of inoculations, conditions were not so favorable for virulent infection. The 6 cowpea isolates were pathogenic on the cowpea. Artificial infection of young cowpea plants by stem inoculation frequently led to their entire destruction without canker formation, but typical fissures resulted on the stem of older plants. Infection was not obtained on the cowpeas when inoculated with *X. phaseoli.*

[1] Dunlap, A. A. Two bacterial diseases in Texas. U. S. Dept. Agr., Bur. Pl. Ind. Plant Dis. Rptr., 27: 274. 1943.

Six months later these experiments were repeated in a similar manner with the exception that the bean blight pathogens were reisolates of those formerly used. By this time the cowpea pathogen had lost to a still greater extent its virulence for the bean. Small cankers appeared on the stem at the point of inoculation, and, occasionally, a leaf would wilt; but the plants did not die rapidly, as they did with recent isolates. The cowpea pathogen, however, still retained its virulence for its own host. No change was noted with the cultures of *Xanthomonas phaseoli.*

Further inoculation experiments were conducted on 3 other leguminous plants; alfalfa, lespedeza, and soy bean. These tests were duplicated but in no case was infection obtained. It, therefore, was evident that the cowpea organism was not any of the following bacteria that it resembles somewhat: *Xanthomonas alfalfae* (Riker *et al.*) Dowson, *X. lespedezae* (Ayers *et al.*) Burkholder, nor *X. phaseoli* var. *sojense* (Hedges) Starr and Burkholder.

The fact that recent isolates of the cowpea pathogen are extremely virulent on Red Kidney beans makes the organism of possible importance to the bean crop. Furthermore, the two pathogens being so similar in appearance, the cowpea organism already might be infecting the bean and isolates when made might be mistaken for *Xanthomonas phaseoli.* For these reasons investigations were undertaken to determine whether the two showed differences in cultural characteristics and biochemical reactions. If there were differences the identity of the pathogen then could be determined in culture.

Six isolates were used in describing the cowpea organism. These were selected from single colonies on dilution plates and originated from different lesions. Further dilution plates then were made to insure purity and each isolate was tested for pathogenicity. All behaved similarly, both pathogenically and in culture. Four isolates of *Xanthomonas phaseoli* (XP1, XP2, XP4 and XP14) were tested at the same time. The first two isolates were old cultures, but the last two were those used in the inoculation experiments reported above, and had been reisolated in January, 1943. A number of differences were found between the two pathogens, as is shown by the following description of the cowpea organism.

Morphology. The bacterium is a rod with rounded ends. In a 48-hour-old culture on beef-extract-peptone agar at 27° C., the size of cells are 1.76 μ (1.0 to 2.8 μ) by 0.7 μ (0.46 to 0.92 μ). The Congo red stain was used in this determination. The bacteria are motile with 1 polar flagellum. They are Gram-negative.

Cultural Characters. On beef-extract-peptone-agar slants at 27° C., a moderate growth develops along the streak in about 48 hours. It is filiform, with edges entire, glistening, primuline-yellow, butyrous. On potato-dextrose agar, growth is more abundant, mucoid, and pale-yellow to colorless. In beef-extract bouillon, a cloudy growth appears in 48 hours. There is a heavy yellow ring on the glass but no pellicle. In litmus milk after a week's growth there is a reduction of litmus and a light curd develops that soon becomes solid and remains so for several weeks. A slow peptonization follows and crystals, presumably tyrosine, are formed in the medium. At the end of 6 weeks the milk is a brownish-purple syrup. In shake cultures of beef-extract-peptone agar plus 0.5 per cent dextrose, colonies appear only on the surface of the medium, showing that the pathogen is a strict aerobe.

The optimum temperature for growth is 27° to 30° C. Maximum 37° C. and minimum between 6° and 9° C.

Biochemical Reactions. Growth in gelatin stabs is good; slight liquefaction begins on the second day and proceeds rapidly. Growth in tryptone broth is excellent and hydrogen sulphide production is evident after 4 days. The tests were made with strips of

filter paper impregnated with lead acetate. The Goré method shows no indole formation. There is a very slight to no growth in synthetic nitrate broth. Nitrites are not formed. In beef-extract-broth plus 0.1 per cent KNO_3 there is good growth but no nitrites at the end of 3, 5 and 10 days. $NH_4H_2PO_4$ can be utilized for nitrogen. There is no growth in Clara's medium[2] nor in the same solution when tyrosine is substituted for asparagine showing that these two amino acids cannot be utilized as a combined carbon and nitrogen source. However, in a richer medium tyrosine appears to be broken down, judging by the color reactions—a pink to a brown. On spirit-blue agar[3] there is a definite lipolytic action. In broth, growth is retarded with 2 per cent NaCl and inhibited with 3 per cent.

The cowpea pathogen grows slowly in a synthetic medium for carbohydrates[4] but after two weeks the following data were obtained. All carbon sources with the exception of the organic acids were filtered. Acid is formed in dextrose, galactose, lactose, maltose, sucrose, and raffinose. There is no growth in levulose, l-arabinose, xylose, rhamnose, glycerol, and salicin. An alkaline reaction is obtained with salts of citric and malic acid, while the growth in acetic acid was irregular. There is no growth in salts of lactic, formic, succinic, tartaric, and hippuric acid. Starch is hydrolyzed.

In the above experiments it was demonstrated that there are certain distinct differences between the two pathogens. Noticeably, the cowpea organism is much slower in growth in all media, and has a tendency to lose its viability in culture. This behavior cannot be accounted for on age of isolate, since XP4 and XP14 of *Xanthomonas phaseoli* were reisolates and they behave like the two older isolates of this species. The greatest difference between the two pathogens is noted in milk. The cowpea organism produces a solid curd that lasts several weeks before peptonization begins. The bean pathogen begins to clear the medium on the second day with no curd formation. The temperature relations also are different; *X. phaseoli* grows at 6° C. but not at 36° C., while the cowpea bacterium does not grow at 6° C., gives a slight growth at 9° C., produces a good growth at 36° C., but none at 38° C. There are some differences in the ability of the pathogens in their energy sources. *X. phaseoli* utilizes xylose, glycerol, succinic acid salts well, and levulose lightly; the cowpea pathogen does not utilize these carbon compounds, but utilizes mannitol, which *X. phaseoli* does not. The experiments from which the above data were collected were repeated with the same results.

Inasmuch as the cowpea pathogen differs definitely from *Xanthomonas phaseoli* in both pathogenicity and cultural reactions, it is considered a new species and the following name is proposed, *Xanthomonas* **vignicola** sp. n.

SUMMARY

A description is given of *Xanthomonas* **vignicola** n. sp., a bacterium that causes cankers on the stems of cowpeas and a blight of the common bean. The pathogen is similar to *X. phaseoli* but differs distinctly in its pathogenicity and in certain cultural characteristics.

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[2] Clara, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. New York (Cornell) Agr. Exp. Stat. Mem. 159: 1–34. 1934.

[3] Starr, Mortimer P. Spirit blue agar; a medium for detection of lipolytic microorganisms. Science 93: 333–334. 1941.

[4] Society of American Bacteriologists, Committee on Bacteriological Technique. Manual of methods for the pure culture study of bacteria. (Geneva, N. Y.) 1923. Leaflet II, ed. 8, 1942, p. 15.

CHARCOAL ROT OF IRISH POTATOES[1, 2]

R. D. WATSON

(Accepted for publication November 17, 1943)

The rotting of Irish potatoes has been a serious disease in East Texas to the knowledge of more than a generation of growers. Most of the rotting occurs in the field after the plants reach maturity and in the early part of the storage period. The primary causal agent appears to be *Sclerotium bataticola* Taub. This fungus has been reported[3] as causing decay of Irish potatoes in the Eastern Mediterranean area and in India. A serious tuber decay of Irish potatoes grown under irrigation in the Texas Panhandle in the summer of 1943 was later found to be due to this same fungus.[4]

All varieties of Irish potatoes grown in this region appear to be susceptible to charcoal rot. The varieties on which it was observed this year were Bliss Triumph, Irish Cobbler, Katahdin, Chippewa, and Rural. Losses often have amounted to 50 per cent or more of the crops and seldom are they below 5 per cent, with an estimated average of 15 to 20 per cent of the stored crop in 1943. The amount of decay is closely correlated with the date of digging and the moisture content of the soil, particularly at digging time. Wet soils and high temperatures favor the development of the disease.

Charcoal rot attacks the maturing plant at the onset of hot weather. It probably infects the seed piece, develops in the plant, and goes down through the stolon to cause the conspicuous rot on the tuber. Early-, medium-, and long-season potatoes as well as early- and late-planted ones died all at about the same time, even though the soil moisture seemed adequate for their growth. This may indicate that this is an important disease of the plant, as well as of the tuber.

The external symptoms on the tuber are typically a blackened and somewhat soft or semi-wilted infected area. The internal symptoms show blackening to various depths, but ordinarily the discoloration is restricted to the outer area of the tuber. The infections are most often at the stolon end and around the eyes (see Fig. 1). During wet weather swollen lenticels also provide points of entrance by the fungus. *Sclerotium bataticola* inoculated into the tuber produced the typical symptoms of the rot whether the isolate came from potato or from cowpeas. The fungus was reisolated from these inoculated tubers.

The later and final stages of the rot are ordinarily associated with one or both of the following organisms: (1) *Erwinia carotovora* (Jones) Holland in which case the tuber breaks down into a typical soft rot but with the

[1] Approved by the Director of the Texas Agricultural Experiment Station as Technical Paper No. 821.

[2] Previous mention of this disease has been made, (Abstract) Phytopath. 33: 1120. (1943) 1944.

[3] Rev. of Appl. Mycol. 9: 561; 10: 437; 11: 126; 17: 552; 19: 198.

[4] A survey of Irish potato diseases in Texas Panhandle fields in August, 1943. Texas Agr. Exp. Stat. Mimeographed Prog. Rept. No. 859.

blackening of the original charcoal rot still evident. This organism was most commonly associated with charcoal rot at digging time or shortly afterwards. (2) A *Fusarium* sp., which with *S. bataticola* produces a water-soaked, spongy rot, and the tuber ordinarily dries into a mummy with a white fungus outgrowth at the eyes or injured portions. This was more common later in storage. The rotting that occurs in storage seems to a large

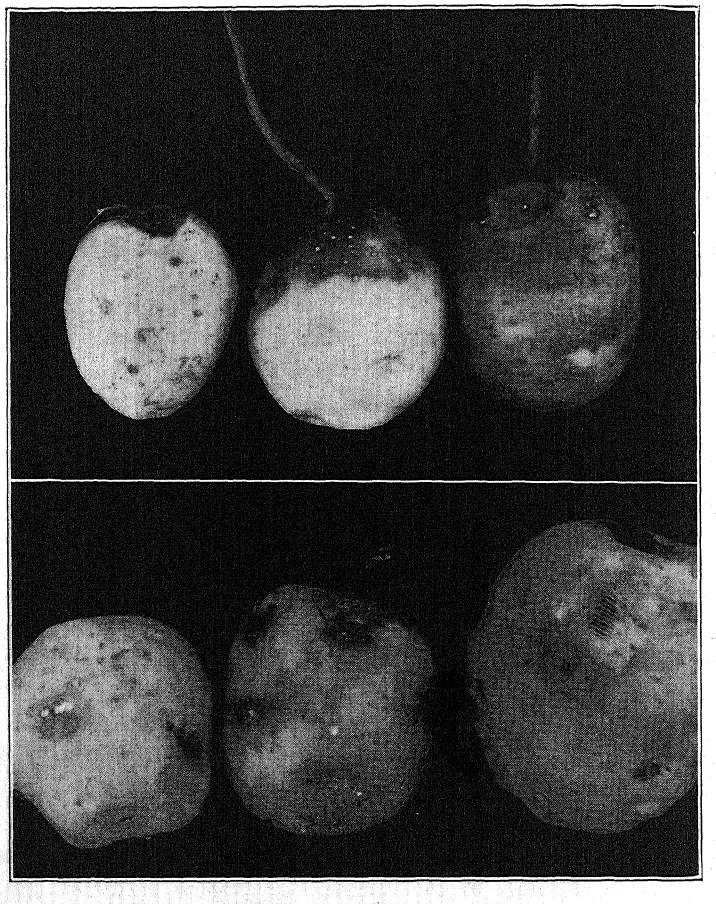


FIG. 1. Above, typical stolon infection of the charcoal-rot fungus on the tubers of Irish potato. Below, eye infection by the charcoal-rot fungus, *S. bataticola*.

extent attributable to infection in the field. Charcoal rot on the tuber somewhat resembles late-blight rot, but the two are disinguishable to one familiar with the latter.

High temperature seems the most important of the environmental factors affecting incidence and severity of charcoal rot. Abundant moisture also favors development of the disease. Although *Sclerotium bataticola* is pathogenic at temperatures below 80 to 85° F., the rotting is slow, and, as the temperature rises, the rate of rotting is very much accelerated. The disease has

been reported on Irish potatoes for the most part from tropical or semi-tropical regions where high soil temperatures during harvest season might be expected. In East Texas the crop is planted early in the spring and matures as summer begins. Undoubtedly the high soil temperatures of summer are responsible for its common and destructive occurrences.

Control recommendations are based on the present knowledge of the disease and its chief epiphytological factors. Since the organism is present in most of the soils in the region and has a wide host range, rotation alone will be of little value. It is important to remove the tuber from the ground before it becomes infected and before high summer temperatures increase the activity of the organism. The use of early varieties, planted and harvested early, aids in avoiding field infection of the tubers. Care must be used in handling these immature tubers to avoid bruises, sunscald, and other injuries that will increase the amount of rot. Only well-drained land should be used. Potatoes should be sorted to remove any early-stage rot and kept dry in crates, which provide for air circulation. Forced circulation will reduce the secondary rots. Cold temperatures of 34 to 38° F. reduce the amount and rate of rot during storage.

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PHYTOPATHOLOGICAL NOTES

Guttation-salt Injury on Leaves of Cantaloupe, Pepper, and Onion.—The formation of salt deposits on the leaves of various plants following guttation, and injuries associated with these deposits, have been observed[1] in the Winter Garden Region of Texas. As early as 1804, De Saussure[2] reported as follows on a certain "maladie blanche" apparently well recognized at that time as a nonparasitic disease on cucurbits and other garden plants:

> . . . the superfluous salts accumulate in certain plants upon the surface of their leaves, and form an incrustation which makes them perish, by preventing transpiration. Such is in large part the origin of the white malady ('maladie blanche'), which attacks the Cucurbitaceae and many garden plants. This malady begins, in the case of the cucurbits, with the viscous drops, which appear principally upon the surface of the leaves in the vicinity of the petiole. These drops dry out and form white conspicuous powdery stains, which extend and multiply successively near the circumference of the leaf. I have removed this incrustation; it is not soluble except in part, in water and in alcohol. These solutions after evaporation and drying presented a deliquescent salt, which had all the properties of calcium muriate united with an inappreciable quantity of magnesia; it was abundantly precipitated by silver nitrate, potassium oxalate, and the alkaline carbonates, but not at all by baryta water, and it was almost unalterable by fire. The saline and the earthy metals' part of the incrustation formed about one-third of its weight; it is enveloped by a white vegetative substance insoluble in water and in alcohol, and sufficiently abundant because the incrustation of the same was not sensitively attracted by the humidity. This malady attacks principally the old plants which grow upon a soil highly saturated with animal manure, and under layers where the leaves have not been washed by the rains.

On the edges of cantaloupe leaves, local necrosis, in the form of small brown semicircular areas, has been observed to develop at the hydathodes following guttation and accumulation of salt deposits at these points. These necrotic spots are frequently bordered, and at times preceded, by a chlorotic area (Fig. 1, A). The spots may coalesce and form a band of necrotic tissue around the entire margin of the leaf. In some cases the whole leaf may die. Cantaloupes may guttate frequently and profusely, but, if the concentration of the salts in the guttation water be low and no large amounts of salts be deposited on the leaf, no injury may occur. The composition of these deposits is not entirely known, but they seem to contain several common soluble salts in addition to calcium carbonate. Similar injury has resulted, both in greenhouse and field, under partly controlled conditions by inducing guttation and heavy salt deposits or by placing guttated salts from Tamarix in the droplets on the cantaloupe leaves.

With the pepper plant, salt deposits have been observed on the surface of the leaves following guttation through the upper surfaces, accompanied by injury in the form of large necrotic areas, often with a water-soaked border (Fig. 1, B and C). This injury has been reproduced in the laboratory by transferring salts from the surface of the necrotic areas to uninjured

[1] Ivanoff, S. S. Chemical deposits on foliage of citrus and other plants and their possible relation to chlorosis and yield. Texas Agr. Exp. Stat. 54th Ann. Rpt., 181–182. 1941.

[2] De Saussure, Théodore. Recherches chimiques sur la végétation. p. 264–265. Paris. 1804. (The quotation given here is a translation from the original French, by the writer.)

pepper leaves in moist chambers. The salts from peppers were very hygroscopic and seemed to contain little or no calcium carbonate.

On onions, amorphous or crystalline white deposits in the form of small aggregates or lumps up to about 1 mm. in diameter have been observed, from few to several hundreds on a single leaf (Fig. 1, D). Some time following their formation, there usually appear specks or spots underneath the deposits. These may be white, pale yellow, or tan, apparently depending on their age. Aggregates of these spots resemble the leaf symptoms of "onion blight," a nonparasitic trouble of economic importance.[3] Similar symptoms, including blighting of onion leaf tips, have been produced experimentally by placing dry guttation salts collected from Tamarix on the young onion leaves, where the salts deliquesced.

Since guttation seems to be a common phenomenon, it is possible that other plants may be subject to this same injury. Possibly, the nonparasitic

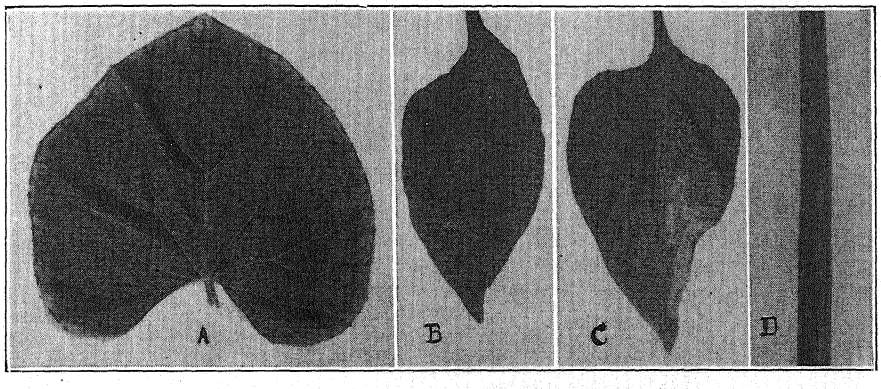


FIG. 1. Guttation-salt deposits and associated injury to leaf tissues. A. Cantaloupe leaf showing numerous semicircular necrotic lesions at the hydathodes (where guttation-salt deposits were previously observed) with nearby chlorotic and necrotic areas. B. Pepper leaf with dry salt deposits following guttation through the upper surface. C. Pepper leaf with guttation-salt injury at the moistened lower portion of the leaf following partial dissolving of salt deposits. D. Onion leaf with salt deposits, beneath which were formed small spots of dead tissue.

tipburns of lettuce, potato, and of other plants sould be re-examined from this standpoint.—S. S. IVANOFF, Texas Agricultural Experiment Station, Substation 19, Winter Haven, Texas.

Sporonema Rot of Apples.—In March, 1936, a York Imperial apple that had been stored at 36° F., was found to have a large decayed area, with numerous pycnidia in the center. The causal organism was identified by C. L. Shear as *Sporonema oxycocci*, a fungus causing decay of stored cranberries and first described by him in 1907.[1] This fungus never has been reported as causing decay of apples, and no further cases of natural infection have been observed. As cranberries were stored in the same room with

[3] Ivanoff, S. S. Onion "blight." Texas Agr. Exp. Stat. 51st Ann. Rpt. 260–261. 1938.

[1] Shear, C. L. New species of fungi. Bul. Torr. Bot. Club 34: 305–317. 1907.

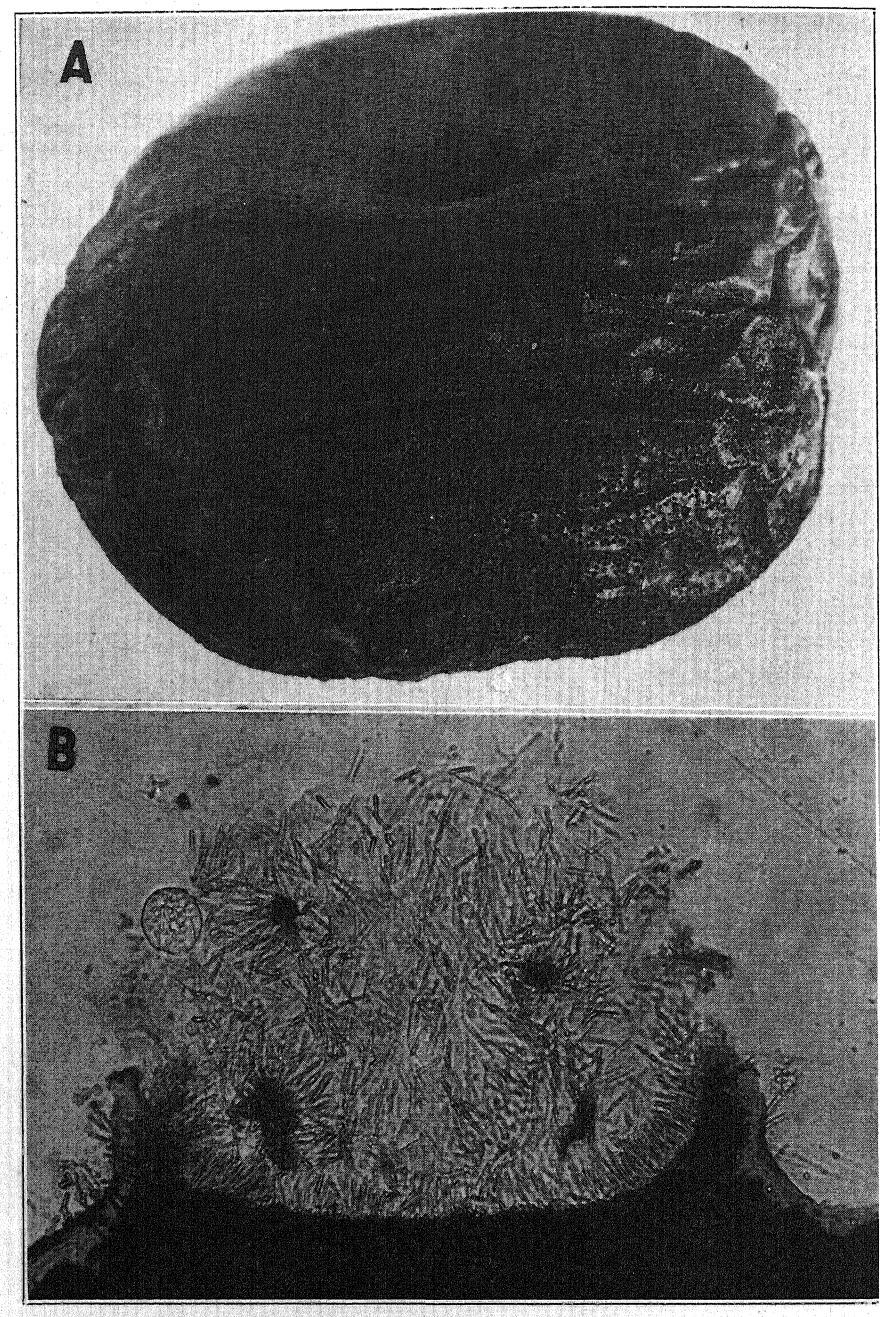


FIG. 1. *Sporonema oxycocci* causing artificially produced rot on York Imperial appl at 50° F. A. Numerous pycnidia protruding through the surface of the decayed tissue B. Cross section of a pycnidium showing the cylindrical spores.

the apples, the decay was noted with interest, but considered as incidental. However, in the course of other studies on the source of certain fungi causing decay of stored apples, a species of *Sporonema* was isolated and was again found to cause decay when inoculated into apples. This experience made it seem worth while to report the susceptibility of stored apples to *Sporonema* and to include briefly certain observations and descriptions.

This fungus was found causing decay in 6 of the 44 inoculations, where small quantities of surface soil taken from a wooded area near an apple orchard were inserted into oblique surface wounds on apples and these were then stored at 31° F. for development.

The disease was produced artificially; typical lesions developed in York Imperial apples that were slightly wounded and dipped in an aqueous pycnospore suspension, and subsequently stored at 31°, 36°, and 50° F. In these tests large, open lenticels also occasionally served as infection courts.

The rot developed slowly at 31° F., but in the course of several months of storage the lesions developed to serious proportions (Fig. 1–A). The decayed tissue was pale-brown at first, but became progressively darker with age until, at the time the pycnidia (Fig. 1–A) were formed, the underlying tissue was quite black. The rot that developed at 50° F. was darker than that at 31° F. Mature pycnidia have not been found at 31° F., but have occurred on apples at 36° F. The pycnidia originate immediately below the decayed peel and become erumpent through it (Fig. 1–B).

The fungus grew readily on Thaxter agar at a wide range of temperatures. After 24 days the average diameters of the colonies as grown at various temperatures on this medium were: 32° F. = 5.8 mm.; 41° F. = 27.6 mm.; 50° F. = 44.0 mm.; 59° F. = 50.8 mm.; 68° F. = 64.8 mm.; 77° F. = 79.5 mm.—L. P. McCOLLOCH, Bureau of Plant Industry Station, Beltsville, Md.

Bacterial Canker of Cowpeas.—The latter part of July, 1942, a grower in Pottawatomie County, Oklahoma, sent to the agricultural experiment station at Stillwater some diseased Whippoorwill cowpea plants for diagnosis. Stems of the diseased plants were characterized by prominent, swollen, cracked cankers. These cankers were located anywhere from the ground level to a height of 6 or 8 inches. Dilution plates from these cankers yielded abundant bacterial colonies and an occasional colony of *Fusarium* sp. A survey of the literature gave no clue as to the nature of the trouble.

In early August, 1942, it was possible to make a personal inspection of the field. The crop was almost a total loss. Only after considerable search was it possible to find what could be termed a healthy plant. In many cases it was noticed that diseased plants had a tendency to break over at the cankered area.

In October, 1942, the same trouble was observed to be present in a mild form in a planting of Chinese Red cowpeas on the Experiment Station plots at Stillwater. The farm foreman stated that the disease had been present in small amounts each year for at least 7 years.

In the 1943 growing season the disease was again noticed at Stillwater and Perkins by early July. By August several varieties in the cowpea nursery at Perkins were badly diseased (Fig. 1). A count of the diseased plants in each 100-foot row gave the data contained in table 1. From this table it is evident that the varieties Wood's Sumptuous, Columbia, Whippoorwill, Early Red, and a few others are susceptible to this disease, while such varieties as Buff or Iron, Victor, Potomac or Calico, Speckled Crowder, Brown Crowder, and several others are either resistant or escaped infection.

During the 1943 growing season the disease has also been observed at other points in Payne County and in Creek, Comanche, and Stevens Coun-

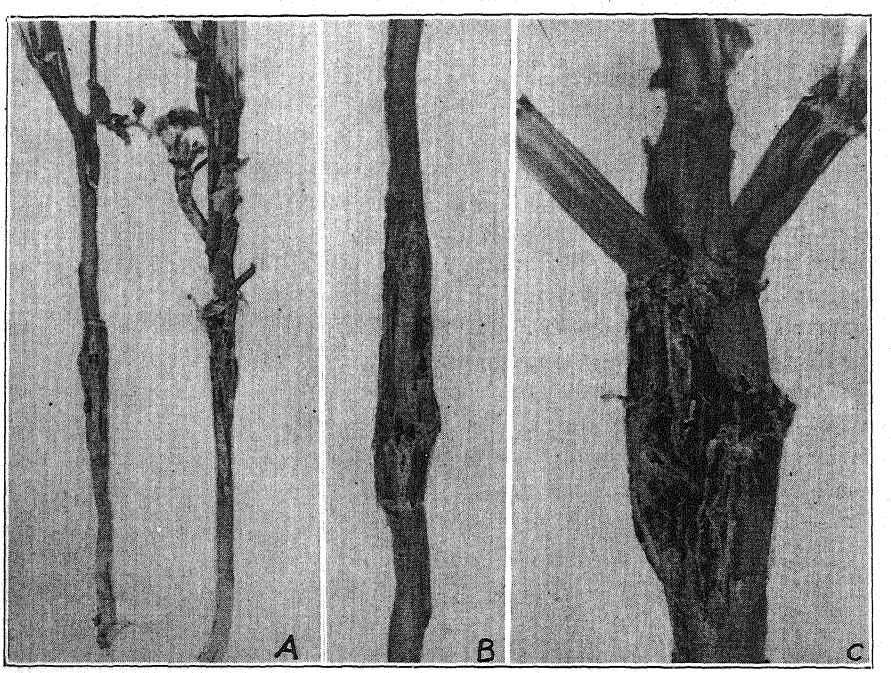


FIG. 1. Bacterial canker of cowpeas. A. Two plants showing the location of the cankers. B. Close up of a typical canker. C. Close up of a badly cankered plant showing the formation of secondary cankers on the branches.

ties, Oklahoma, and near Stuttgart, Arkansas. The disease also appears to have been recently observed in Texas.[1]

According to the unpublished thesis of E. W. Brillhart, in the Botany and Plant Pathology Department at Oklahoma Agricultural and Mechanical College[2] the first authentic record of this disease in Oklahoma is that reported by him from Perkins in 1931. The description and photographs in the thesis leave no doubt but that it is the same canker-producing disease that has been observed during the past two summers. Brillhart states that it may attack leaves, stems, pods, and seeds, but that the most conspicuous

[1] Dunlap, A. A. Some notes on newly observed or unusual diseases. U. S. Dept. Agr. Plant Dis. Rptr. 27: 274. 1943.

[2] Brillhart, E. W. Bacterial canker of cowpeas. An unpublished thesis. Okla. A. and M. College. 1934.

symptom is the stem canker. He indicates that the causal organism is *Bacterium vignae,* which he isolated from diseased leaves, seed, and cankers. He even isolated the bacterium from cankers that had overwintered in the field. Thus it would appear that the organism may overwinter in both the seed and the soil. Brillhart was able to produce typical leaf symptoms in the greenhouse with bacteria isolated from leaves, seed, and cankers of dis-

TABLE 1.—*Bacterial canker of cowpeas. Number of diseased plants per three 100-foot row replicates. Perkins, Oklahoma, 1943*

Variety	Replication			Total
1. Wood's Sumptuous	235	433	152	820
2. Columbia	183	39	38	260
3. Early Red	90	98	33	221
4. Whippoorwill	88	31	102	221
5. Dixie Queen	36	68	70	174
6. Early Ramshorn Blackeye	86	17	38	141
7. Giant Resistant Ramshorn Blackeye	9	65	53	127
8. Clay	4	59	28	71
9. Arlington	8	27	22	57
10. Large Virginia Blackeye	2	29	17	48
11. Chinese Red	4	11	29	44
12. Holstein	15	10	13	38
13. Cream or White Crowder	6	9	16	31
14. Blacks	6	7	17	30
15. Red Ripper	5	2	14	21
16. Virginia Blackeye	0	11	10	21
17. Blue Goose	10	4	5	19
18. Rice or Lady	16	1	2	19
19. Lady Edible Cowpea	2	4	9	15
20. Groit	2	0	5	7
21. Brown Crowder	1	0	3	4
22. New Era	0	3	1	4
23. Braham	0	0	3	3
24. White Browneye Crowder	0	0	3	3
25. Brown or Sugar Crowder	0	0	2	2
26. Potomac or Calico	0	0	1	1
27. Speckled Crowder	0	1	0	1
28. Buff or Iron	0	0	0	0
29. Victor	0	0	0	0

eased plants. The bacterium was reisolated from these inoculated plants. However, he does not specifically state that he was able to reproduce the cankers under greenhouse conditions.—DONALD E. HOFFMASTER, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.

Bibliography and Nomenclature of Puccinia oryzae.—This fungus, apparently a distinct race of *Puccinia graminis* P., is known only from limited areas in Spain and Italy[1] where scant attention has been accorded it because under the peculiar ecological conditions and crop practices there encountered it is ordinarily not an economic menace. It has, however, such destructive potentialities that plant quarantine measures against it elsewhere are abundantly justified. The chief references in scientific literature to this pathogen are unfortunately conspicuous only for their

[1] It is significant that what is doubtless this same fungus has been recorded also from Roumania by O. Savulesco (p. 305): Micromycètes trouvés sur le riz en Roumania—Acad. Roumaine, Bull. Sect. Sci. 22 (no. 7): 305–308. 1940.

bibliographic shortcomings. They are commented upon in chronological order, quoting the nomenclature employed in each instance as follows:

1903. Briosi, G. Rassegna crittogamica pel primo semestre 1903—in Bollet. Uffi. Minist. d'Agr., Ind. e Comm. N.S. Anno II, 4: 83–95 fasc. 203 (Rome, Italy).—Briosi on pages 84–85 discusses the symptoms of the disease and the characteristics of the pathogen, which he calls "*Puccinia oryzae*" without, however, labeling it as a newly named species, omitting a technical description, yet, without reporting any previous observations of it. This particular contribution of Briosi's was probably printed and distributed as a separate and it has most likely been confused, as will be pointed out further on, with another paper having a similar title, which did not contain any reference to his *Puccinia oryzae*, namely: "Rassegna crittogamica pel primo semestre 1903" in Att. Ist. Bot. Pavia 2 ser. 9: 323–339, (Milano) 1911.

1908. Trotter, A. Flora Italica cryptogamica Pt. 1, fasc. 4 (Uredinales), pp. 519.—On p. 477 Trotter notes a "*Puccinia oryzae*" upon rice as "Riso in Lombardia (Bibl. 263 1 sem. an. 1903)." He did not cite any author for the species, although by his reference it should be Briosi. The citation involves difficulties since by "Bibl. 263" is meant no. 263 of Traverso's Bibliography in Fl. Ital. Crypt. Pt. IV, fasc. 1, which is: "263) 1886–1903—Briosi, G.—Rassegna crittogamiche. Milano (in At. Ist. Bot. Pavia vol. I–VIII in 8°)." The article by Briosi for "1 sem. an. 1903" does not, however, appear in vols. I–VIII as indicated by Trotter but as cited above in Att. Ist. Bot. Pavia vol. 9, 1911. Although in the main duplicating information contained in the 1903 report by Briosi first cited above, it omits entirely any mention of *Puccinia oryzae* or of *Puccina graminis* upon *Oryza*, showing that Trotter failed to realize that the two papers by Briosi were not identical.

1909. Sydow, P. & H. Monographia Uredinearum 1: pp. 972.—This monograph on p. 695 records *Oryza sativa* as one of the hosts for *Puccinia graminis* P. but does not note *P. oryzae* or any special rice form or variety of the parasite, nor does it cite Briosi's account.

1915. Florensa y Condal, Jose. Sindicato de Riegos del Delta del Ebro, la Infermedad del Arroz, Terragona pp. 32, ill. (abstr. in Mo. Bull. Agr. Intell. & Pl. Dis. Int. Inst. Agr. (Rome) 6: 460–470, 1915 no. 3.) (abstr. in Techn. Rundschau 7: 514–515, 1915).—This is a semi-popular account of the rust fungus here named *Puccinia oryzae* (author not noted) in connection with a seriously diseased condition of rice in a rice-growing area of Spain.

1918. Gonzales Fragoso, R. Enumeraçion y distribuçion de las Uredales . . . Iberica e Islas Baleares. Trabajos Mus. Nac. Cienc. Nat. Ser. Bot. Num. 15: 5–267.—The fungus is here referred to (p. 39) as "*Puccinia graminis* P. f. *oryzae* (Risso) in Rass. critt. Padova 1 sem. an. 1903—Trotter, Ured. de la fl. ital. p. 477." The author had doubtless misunderstood the meaning of "riso," the Italian word for rice, in Briosi's account, possibly confusing it with the name "Risso" which he might have taken to be a reference to Antoine Risso (1777–1845) a botanist of an earlier period who appears to have had no connection whatever with the name of this fungus on rice. It is obvious that he also confused Padua with Pavia.

1919. Oudemans, C. A. J. A. Enumeratio systematica Fungorum 1: pp. 1230.—On p. 723 *Puccinia graminis* P. is recorded as upon *Oryza sativa*, citing in addition to the Sydow Monographia (l.c.) Persoon's "Disp. meth." 1797 and Persoon's "Synops." 1801, although in neither of Persoon's compendia is there any reference to *Oryza* as a host for the species.

1924. Gonzales Fragoso, R. Flora Iberica Uredales, pp. 424.—The rice fungus is here (p. 26) considered under the name "*Puccinia graminis* P." without further elaboration.

1882–1931. Saccardo, P. A., *et al.*—Sylloge Fungorum vols. 1–25. A reasonably diligent search of the indexes to these 25 volumes has failed to reveal there any citation to this fungus as upon *Oryza*, unless so noted under the abbreviation "etc."

The name *Puccinia oryzae* having no technical description is clearly a nomen nudum, but as treated by Gonzales Fragoso (l.c., 1918) it would be valid as *Puccinia graminis* P. f. *oryzae* Frag.—WILLIAM W. DIEHL, Bureau of Plant Industry Station, Beltsville, Md.

BACTERIAL WILT OF TOMATO CAUSED BY PHYTOMONAS SOLANACEARUM[1]

EDWARD K. VAUGHAN[2]

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INTRODUCTION

Each season millions of tomato seedlings are shipped from the South to the Middle Atlantic and Middle Western States. Occasional fields set with these plants suffer severely from bacterial wilt infection. In some instances the disease develops soon after the plants are set out, while in other instances the plants appear to be quite healthy until as late as July or early August and then succumb to the disease. While representatives of the southern growers, the northern farmers, the canning companies, and the various experiment stations generally have agreed that the early-season infections take place in the South, there has been a great diversity of opinion concerning the infections that appeared later in the season. It seemed desirable, therefore, to determine (a) whether infected plants might appear to be perfectly healthy; (b) whether apparently healthy plants might grow vigorously for several weeks and then succumb to an infection which had taken place during the early seedling stages; and (c) whether the organism, *Phytomonas solanacearum* (E.F.S.) Bergey *et al.*, once established in Northern soils, could survive the winter and infect subsequent crops of tomatoes grown on those soils. For the benefit of the southern grower it also seemed desirable to determine the influences of soil temperature, moisture, and hydrogen-ion concentration on infection by the wilt organism.

HISTORICAL REVIEW

Bacterial wilt of solanaceous plants was first noted by Burrill (1) in 1890 and was described by Smith (10) in 1896. Since that time investigators in many countries have endeavored to control the disease. Smith (11, 12) suggested that infection could be reduced by controlling insect carriers of the disease, particularly the Colorado potato beetle, and that cultivation practices that would minimize root injury would help to prevent infection.

[1] The field data and a portion of the laboratory studies presented in this paper were conducted as a portion of cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dept. of Agriculture, and the Georgia Coastal Plain Experiment Station, Georgia Agricultural Experiment Station, Georgia Dept. of Entomology, New Jersey Agricultural Experiment Station, and Indiana Agricultural Experiment Station. The work here reported is part of a thesis presented to the Faculty of the Graduate School of the University of Minnesota, in part-fulfillment of the requirements for the degree of Doctor of Philosophy; August, 1942.

The writer wishes to express his sincere appreciation to Dr. S. P. Doolittle and Dr. W. D. Moore for invaluable advice in planning these investigations and for assistance in carrying on many phases of the work. He also wishes to thank Dr. S. A. Wingard, Dr. G. M. Shear, Dr. I. D. Wilson, Dr. E. C. Stakman, Dr. C. J. Eide, and Betty V. Conner for assistance in preparing and editing the manuscript.

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Nakata (6) reported that in Japan *Phytomonas solanacearum* grew only in soils with a reaction between pH 6.0 and 8.1, but Eddins (3) was able to obtain growth in culture on agars having reactions between pH 4.25 and 8.71. He found also that the organism was present in Florida soils having reactions as low as pH 4.32. He was able to control the disease, however, by treating soils with sufficient inoculated sulphur to reduce the pH to between 3.5 and 4.0.

Earle (2), van der Meer (4) and others have reported that an increase in the moisture content of infested soils results in an increased infection by *P. solanacearum,* although Nakata (6) found that there was a "detrimental water content" of the soil which would kill the organism. Meier and Link (5) found that temperatures between 77° F. and 97° F. were optimum for the development of bacterial wilt, and that the disease is inhibited at temperatures below 55° F.

METHODS AND MATERIALS

Except when other methods are mentioned, all inoculations were made by removing the plants from the soil in which they were growing, washing the soil from the roots, and then soaking the roots in a suspension of the bacteria for 5 to 15 minutes, after which the plants were reset before the roots were dry. All cultures were kept on 1.8 per cent potato-dextrose agar, and two-day-old cultures were used for transferring to test media. Most suspensions for inoculating plants were made by growing the bacteria in potato-dextrose broth for two days, although such suspensions have no apparent advantage over water suspensions of bacteria from agar cultures.

Before plants from the field or in the greenhouse were reported as being infected by *Phytomonas solanacearum* the diagnosis was confirmed by microscopic examinations; and, in many instances, also confirmed by isolating the organism and growing it in pure culture.

Source of Phytomonas solanacearum Cultures

The source of each culture used in these studies is shown in table 1. Because of the tendency, noted by Smith (12) and others, for *Phytomonas solanacearum* to die in agar cultures, the different strains were maintained in plants growing in pots of sterilized soil. Even under these conditions the organism frequently died or lost its pathogenicity, so that new cultures had to be secured from time to time. Several other cultures were used, but only those mentioned in the text are listed in table 1.

TABLE 1.—*Source of Phytomonas solanacearum cultures*

Number	Place of collection	Date of collection	Isolated by
96	New Brunswick, N. J.	August 8, 1938	E. K. Vaughan
98	Freehold, N. J.	August 17, 1938	E. W. Geigel
98x	Freehold, N. J.	August 17, 1938	E. W. Geigel
103	Moultrie, Ga.	April 28, 1939	E. K. Vaughan
151	Tifton, Ga.	February, 1942	W. D. Moore

Selection of Apparently Healthy Plants

Practically all of the tomato plants shipped to northern states from the South are inspected at various times during the growing period by state certifying agencies or by representatives of the northern plant brokers. Fields in which bacterial wilt is found are rejected, and it is doubtful whether many plants showing visible symptoms of the disease are ever shipped. Certification regulations require an interval of two years between tomato crops, and no other solanaceous crop may be grown during this period. Despite the precautions taken, a number of fields each year suffer from wilt infection, which cannot be traced to previous crops of susceptible plants.

TABLE 2.—*Development of bacterial wilt in tomato plants from infested and non-infested fields, when planted at New Brunswick, New Jersey, in 1940*

Lot No.	Number of plants set June 4	Number of plants showing symptoms of bacterial wilt		Per cent infected by wilt
		June 17	July 30	
Infested field				
1	26	1	1	3.8
2	26	1	2	7.6
3	24	6	20	83.3
4	23	7	20	86.9
5	26	0	0	0.0
6	25	2	5	20.0
7	24	14	21	87.5
8	26	4	3	11.4
Total	200	37	72	36.0
Non-infested field				
1	23	0	0	0.0
2	23	0	0	0.0
3	25	0	0	0.0
4	26	0	0	0.0
7	26	0	0	0.0
8	26	0	0	0.0
Total	199	0	0	0.0

In 1939 the plants in several badly diseased fields in New Jersey and Delaware were traced to a definite field in the southern plant-growing area. This field had been carefully checked at the time the plants were pulled, and only an occasional wilted plant was found. The northern growers reported that the plants appeared to be in excellent condition when they were received, but that the disease appeared soon after they were set out. In the spring of 1940, experimental plantings were again made in the field under suspicion and also at the Georgia Coastal Experiment Station in soil in which there had not been any bacterial wilt. Apparently healthy plants were selected from both of these plantings and shipped to New Brunswick, New Jersey, where they were set in soil the history of which showed no record of bacterial-wilt infestation. Only the strongest, healthiest plants in each lot were transplanted and each received one-half pint of water at the time of

setting. As shown in table 2, most of the lots from the field where *Phytomonas solanacearum* was known to occur suffered considerable losses because of the wilt. It is possible that the plants that developed wilt later in the season may have become infected from their diseased neighbors rather than in the southern plant field. Nevertheless, the early-season wilt, which could not have been contracted in the northern field, was of serious proportions.

These results indicate: (a) that fields in which there is a considerable amount of wilt one season should not be used for tomato-plant production the following season; (b) that apparently healthy plants may carry the causal bacteria on their roots or that there is an incipient infection that cannot be detected, and (c) that it is unsafe to use plants from fields where the disease is known to occur, even though the plants appear to be free from infection. It is interesting to note that plants from the same seed source, shipped and handled by the same people and in the same manner, but grown in the experimental plots at Tifton, Georgia, did not develop bacterial wilt at any time during the season.

OVERWINTERING OF PHYTOMONAS SOLANACEARUM IN THE SOIL

Although it was known that *Phytomonas solanacearum* lives in the soil from one season to another in the Southern States, and although Smith (11) has reported that it can be frozen in liquid air for 20 hours and yet resume its motility when thawed, it has generally been assumed that the organism cannot survive the winters as far north as New Jersey. From time to time, however, there have been outbreaks of the disease that could not be attributed to infections contracted in the South. In 1939 an outbreak occurred in the experimental plots at New Brunswick, New Jersey, in the same place where the disease was known to have been present in 1938. Since the plants were southern grown it was not possible to state definitely that the organism had lived through the winter in the soil of the experimental plot.

In order to determine whether the bacterial-wilt organism could survive the winter in the soils of New Jersey, 4 fields were selected for study. Near Riverton, a field was selected in which there had been heavy natural infestation in 1939. In fields at Old Bridge, Leesburg Prison Farm, and the New Jersey Agricultural Experiment Station, respectively, heavy infestations were induced by artificially inoculating tomato plants during the 1939 season.

The winter of 1939–40 was unusually severe, and it seemed unlikely that the bacteria would be able to survive if temperature is a limiting factor. In the spring of 1940 these 4 fields were set with Marglobe tomato plants grown from New Jersey certified seed in steam-sterilized (autoclaved) soil. Frequent observations revealed that all of the plants remained wilt-free until the soil temperature remained at or above 70° F. for several hours at a time. As soon as the soil was warm enough, wilt appeared in all 4 of the infested fields. Plants from the same source planted in autoclaved soil remained free from wilt throughout the season (Table 3).

TABLE 3.—*Survival of Phytomonas solanacearum in fields in New Jersey known to have been infested the previous season*

Location of field	Number of plants set	Per cent of plants wilted
Old Bridge	52	8
New Jersey Exp't. Station	600	20
Riverton	72	53
Leesburg Prison Farm	60	85
Autoclaved soil	50	0

In 1941 southern-grown plants set in the experimental plots at New Brunswick again were severely damaged by bacterial wilt. At the Leesburg Prison Farm tomato seed was sown in the infested soil and a large percentage of the plants produced contracted the disease. These results prove that *Phytomonas solanacearum* is capable of overwintering in the soil in most parts of New Jersey even during exceptionally severe winters and of infecting subsequent crops of susceptible plants.

SPREAD OF PHYTOMONAS SOLANACEARUM IN THE FIELD

Alternate rows of plants were inoculated in 1939 at the Leesburg Prison Farm, leaving every other row of plants uninoculated. All of the plants

Condition of plots August 1, 1939

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Condition of plots July 22, 1940, earliest date on which disease was noted

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Condition of plots August 23, 1940, date when final observations were made

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FIG. 1. Overwintering and spread of *Phytomonas solanacearum* in a field at Leesburg Prison Farm, Leesburg, New Jersey. Plants hypodermically inoculated with culture #96, July 1, 1939. O, healthy plants. X, wilted plants.

were left in the field during the winter and the soil was not cultivated or disturbed in any way. In the spring of 1940 the old plants were pulled up and replaced in each case with a healthy seedling.

When the first wilted plants were noticed in this field on July 22, most of them (19 out of 21) were found in the rows where the plants had been inoculated the previous season (Fig. 1). As the season advanced more and more wilt developed in the intervening rows until almost as many of the plants in these rows were infected as in the inoculated rows. The plants in only 3 rows were inoculated in 1939 and it is interesting to note that the plants in the three alternating rows adjacent to these were heavily invaded by the pathogen, while almost none of the plants in the rows that were at a greater distance from the inoculated ones developed the disease. The plants were set 4×4 feet apart and in most cases the organism did not attack plants more than 4 feet from the original place of infection.

At New Brunswick, where only alternate rows of plants were inoculated in 1939, but where the usual plowing and cultivating operations were carried on, there was no difference in the amount of wilt infection that appeared in the various rows in 1940. All cultivating was parallel with the rows, and tomatoes planted in an adjacent plot in both 1940 and 1941 did not become infected, although plants in the inoculated plot only a few feet distant were severely attacked in both seasons. These limited observations indicate that *Phytomonas solanacearum* does not spread rapidly from plant to plant but is dependent almost entirely on the movement of infested soil or infected plant parts or drainage water from one section of a field to another. This is further substantiated by observations made in several commercial fields over a period of 4 years. Moreover, Thomas (14) noted that wilt infections that occurred in low sections of fields in Indiana could frequently be traced to infections in higher parts of the fields. Apparently heavy rains washed the soil from the vicinity of the wilted plants to the lower parts of the field. In such instances the parts of the field adjacent to the washed areas do not become infested, the infections developing only in the flooded areas.

FACTORS INFLUENCING INFECTION BY PHYTOMONAS SOLANACEARUM

Effect of Soil Temperature

Repeated inoculation of potted plants in the greenhouse at the New Jersey Agricultural Experiment Station did not produce the disease. To determine whether this failure was due to low soil temperature, and to determine the effect of soil temperature upon the development of bacterial wilt, the following experiments were carried on at the United States Bureau of Plant Industry Station, Beltsville, Maryland.

On January 8, 1940, 3 series of 10 plants each were inoculated by dipping the roots in suspensions of bacteria or by injecting the suspensions into the stems of the tomato seedlings. The plants were then set in tanks of soil and held at temperatures of 60, 70, 80, 90, and 100 degrees F. With the excep-

tion of one series no wilt developed in any of the series of inoculated plants when the soil temperature was less than 70° F., and very little wilt developed at that temperature. In general, the amount of infection and the rate at which the disease developed, increased with a rise of temperature above 70° F. This general trend was noticeable, even in the plants inoculated hypodermically 2 inches above the surface of the soil (Fig. 2 and 3).

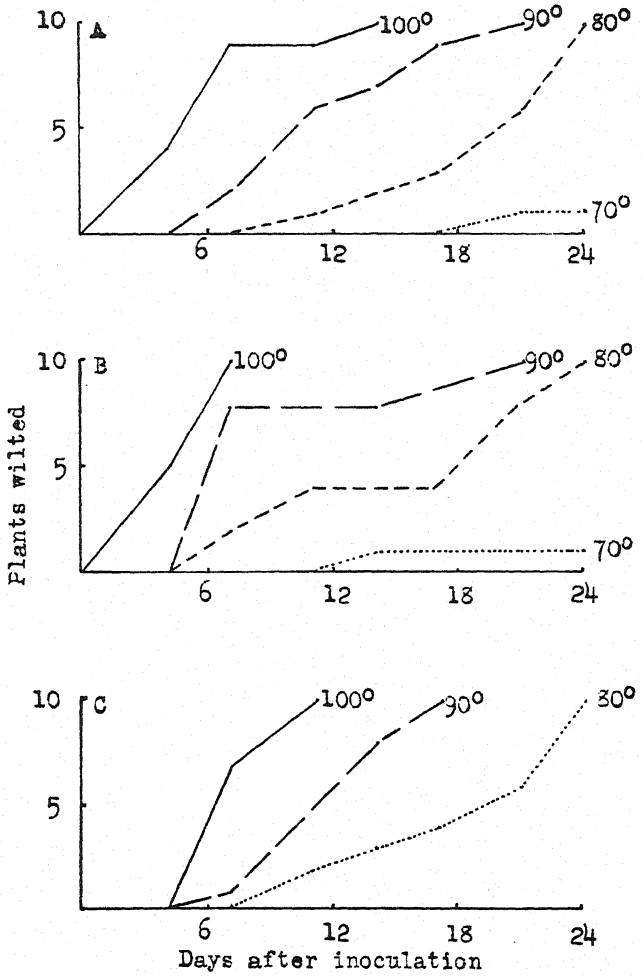


FIG. 2. Development of bacterial wilt in tomato plants inoculated by dipping the roots in a bacterial suspension and planted in soil held at various temperatures. Tests with 3 cultures. A. Culture No. 98. B. Culture No. 98–X. C. Culture No. 103. Ten plants in each treatment. No wilt developed at 60° F. with any culture and none at 70° with culture 103.

Another series of inoculations was made on February 29, 1940, by dipping the roots of tomato seedlings in a bacterial suspension. The 24 plants in each lot were set in tanks of soil kept at 70, 80, 90, 100, and 110 degrees F. Plants in soil held continuously at 110° F. did not grow well. As in the

previous experiments, however, the rate of wilt development increased with an increase in soil temperature above 70° F. (Fig. 4).

Since the critical temperature for wilt development appeared to be about 70° F., plants inoculated on March 28, 1940, by dipping the roots in a bacterial suspension were set in soil held at 60, 65, 70, 75, and 80 degrees F. While the percentage of infected plants (Fig. 5) was not so high as in the previous experiment, the results show that the amount of wilt infection increases with

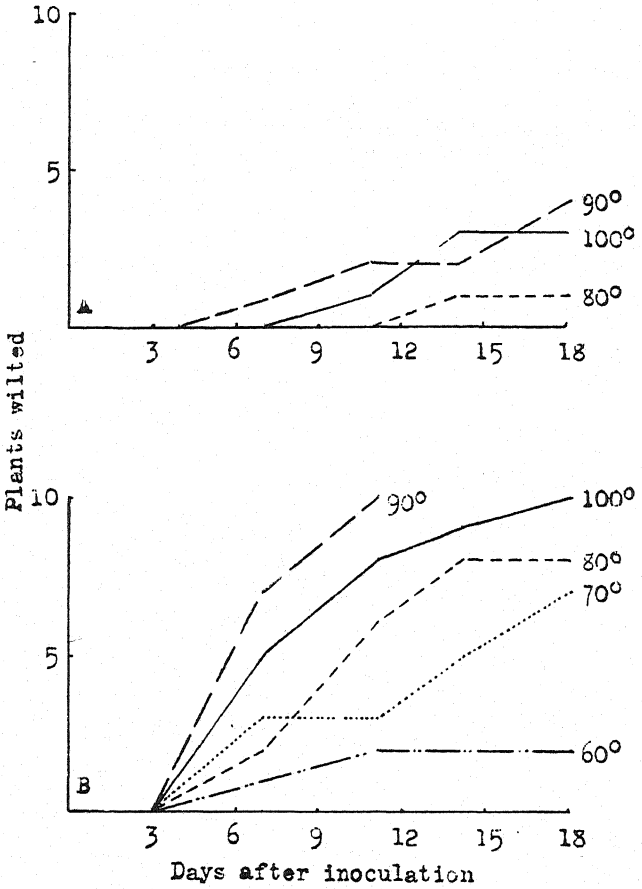


FIG. 3. Development of bacterial wilt in tomato plants inoculated hypodermically and planted in soil held at various temperatures. Test with 2 cultures. A. Culture No. 98, no wilt developed at 60° or 70° F. B. Culture No. 98–X. Ten plants in each treatment.

increased soil temperatures above 70° F. Although no wilt developed in the plants growing in soils maintained at temperatures of 60° and 65° F., a small amount developed in those growing in soils maintained at 70° F. Above that temperature the amount of wilt increased rapidly.

These results, which are similar to those of Meier and Link (4), were further substantiated by field observations. It was noted in the studies on overwintering of *Phytomonas solanacearum* in New Jersey that no wilt

appeared on plants in any of the plots in 1940 until the temperature of the soil at a depth of 6 inches, as recorded by soil thermographs, had remained above 70° F. for several days. In traveling about the State in the spring and early summer frequent readings were made with a soil thermometer. In no case was bacterial wilt observed in a locality where the soil temperature at a depth of approximately 5 inches was less than 70° Fahrenheit.

Since there was no manifestation of the disease when soil temperatures were low, it seemed desirable to determine whether infection did not take place at temperatures below 70° F., or whether infection occurred without producing the characteristic symptoms of the disease. One lot of tomato seedlings, inoculated by dipping the roots in a bacterial suspension, was

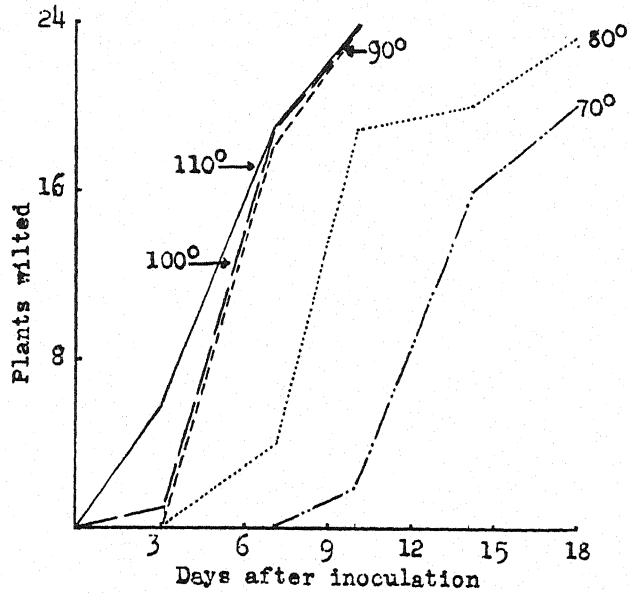


FIG. 4. Development of bacterial wilt in tomato plants inoculated by dipping the roots in a bacterial suspension and planting in soil kept at various temperatures. Culture No. 98. Twenty-four plants in each treatment.

planted in sand and kept at 75° F., a temperature at which wilt developed readily, and another lot in sand was kept at approximately 55° F., a temperature too low for the development of the disease. After 3 days both lots were removed from the sand and the roots and the bases of the stems were washed in a 1 : 3000 solution of New Improved Ceresan for 3 minutes to kill any bacteria that might be adhering to the outside of the plant.[3] After rinsing in sterile water, they were planted in steam-sterilized soil kept at a temperature of approximately 60° F. Some of the plants in each lot did not survive the Ceresan wash, but most of them soon recovered from it. As soon as the plants had recovered from the effects of the transplanting and the Ceresan wash, the temperature of the soil was raised to approximately 80°

[3] Previous tests had shown that this treatment gave 100 per cent disinfection of the root surface of plants taken directly from the soil.

F. and maintained at that point for 3 weeks. At the end of this period 53 per cent of the plants that had been kept at 55° for 3 days following the inoculation had developed the disease, compared with 78 per cent of those that had been kept at 75° for the same length of time.

These results indicate that infection can occur at a temperature of approximately 55°, although infection may take place more readily at temperatures of 70° F. and above. It seems then that plants may become infected at comparatively low temperatures, but that the symptoms of the disease do not develop unless the soil temperature remains for several days at 70° F. or higher.

Plants that had been inoculated and were severely wilted when growing in sand maintained at a temperature of approximately 80° "recovered"

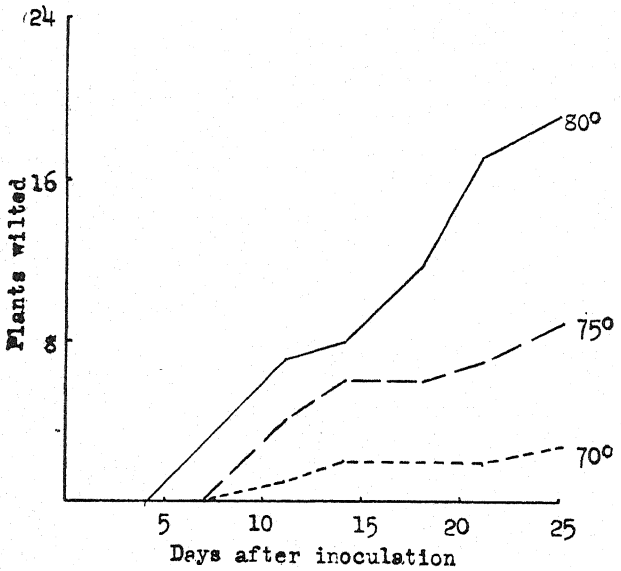


FIG. 5. Development of bacterial wilt in tomato plants inoculated by dipping the roots in a bacterial suspension and planting in soil kept at various temperatures. Culture No. 98. Twenty-four plants in each treatment. No wilt developed at 60° or 65° F.

when the temperature of the sand was lowered to approximately 55° F. for 5 days. When the temperature of the sand was again raised to 80° the plants wilted very quickly, and when the temperature was again reduced to 55° they soon "recovered." This apparent "recovery" took place from 3 to 6 times in different lots of plants before permanent wilting occurred. Infected plants in the field frequently appear to have recovered when examined in the early morning, but wilt again during the heat of the afternoon. This "recovery" may occur several times before the plant finally becomes permanently wilted. This wilting in the field probably is attributable in part to increased transpiration as the air temperature rises, but the phenomenon can be duplicated in the greenhouse by merely raising and lowering the temperature of the soil.

Effect of Soil Moisture

The literature on bacterial wilt contains many references (2, 11, 12, 14) to the fact that the disease causes greater damage in very moist soils than in drier, better drained soils. In order to gain more definite information on the effect of soil moisture upon infection by *Phytomonas solanacearum,* six

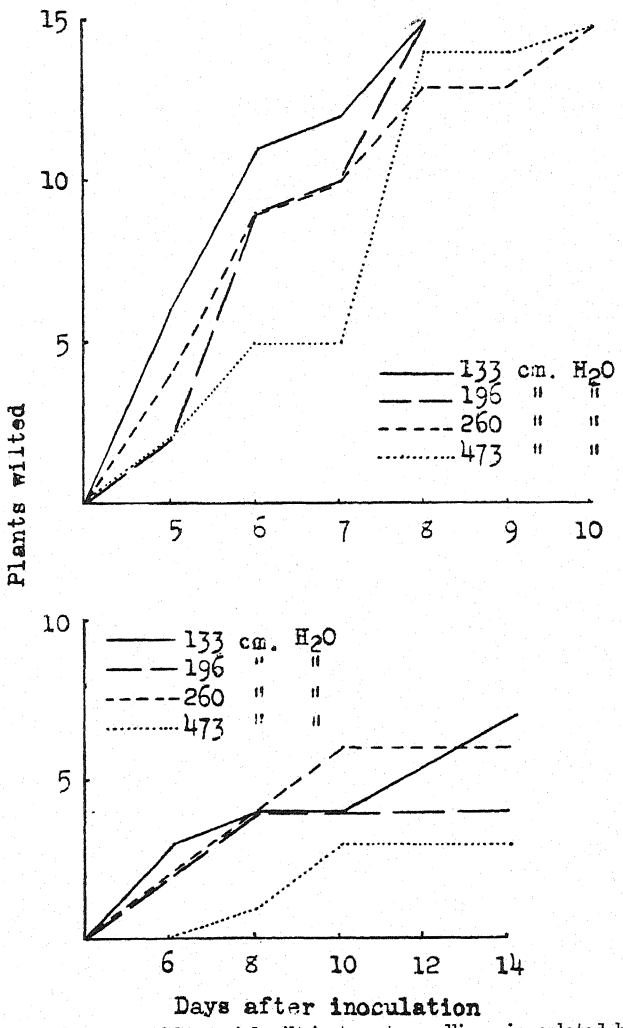


FIG. 6. Development of bacterial wilt in tomato seedlings inoculated by dipping the roots in a bacterial suspension and planting in auto-irrigator pots of soil maintained under different tensions. All pots kept at 84° F. Fifteen plants per treatment in the first test, 10 in the second.

auto-irrigation pots (8) were set up under each of the following tensions: 133, 196, 260, and 473 cm. of water. For the greater tensions, columns of mercury were used in place of water because of the limited amount of available space. Although the differences should have been greater, actual soil moisture determinations showed 21.26, 22.20, 18.48, and 16.40 per cent by

weight, respectively, at the beginning of the first inoculation test and 19.40, 14.40, 13.33, and 10.52 per cent, respectively, at the end of the second test. The pots were kept at a temperature of 84° F. and the soil in all was as uniform as thorough screening and mixing could make it. Fifteen plants in the first test and 10 in the second were set in the soil immediately after being inoculated by root dipping and when the soil had been pressed firmly around the roots the surface of the soil was covered with about one-half inch of glass wool to prevent evaporation.

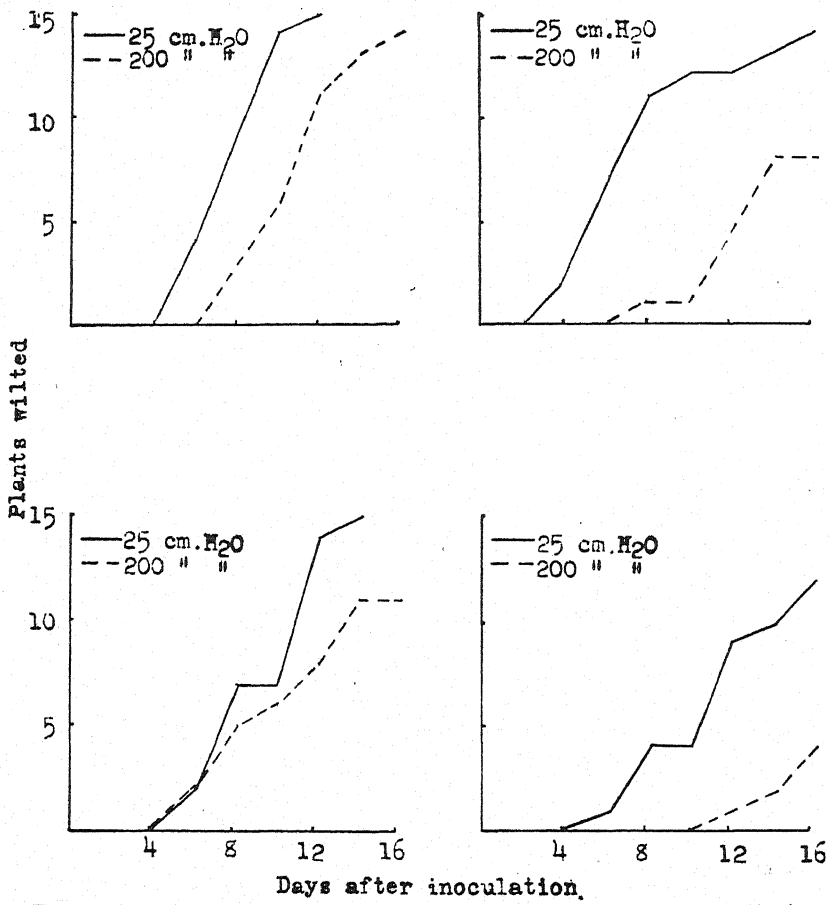


FIG. 7. Development of bacterial wilt in tomato plants inoculated by dipping the roots in a bacterial suspension and planting in auto-irrigator pots of soil maintained under different tensions. Four tests. Fifteen plants per treatment. All pots kept at 84° F.

The results of this test (Fig. 6) are interesting because they indicate that although bacterial wilt does develop more rapidly in moist soils it also may develop in comparatively dry soils, so that the ultimate damage may be equally as great in dry soils as in wet soils.

In later experiments only two tensions were used: 25 and 200 cm. of water. Determinations of the moisture content of the soil at the start of

the first inoculations were 38 and 18 per cent by weight, respectively, at the start of the third test they were 41 and 19 per cent, and at the end of the fourth test they were 36 and 16 per cent, respectively. Again the pots were maintained at a temperature of 84° F. ± 1°. The results of these tests (Fig. 7) are in general agreement with those of the first tests.

Because of the small numbers of plants in each test, it seems inadvisable to draw definite conclusions. There are indications, however, that while *Phytomonas solanacearum* is able to cause infections most readily in fairly moist soils it also can cause infections in comparatively dry soils if the percentage of moisture remains fairly constant. Where soils remain very dry, or are usually very dry except for short periods following rains, infection is not likely to occur.

Effect of Hydrogen Ion Concentration on Growth of Phytomonas solanacearum

Potato-dextrose agar was adjusted to various hydrogen-ion concentrations by the addition of HCl or NaOH to the autoclaved media, using a

TABLE 4.—*Growth of Phytomonas solanacearum on potato-dextrose agar of various hydrogen-ion concentrations*

pH of medium	Culture No. 98	Culture No. 151
4.1	–	–
4.6	–	+
4.9	+++	+++
5.4	+++	++++
6.0	++++	+++++
6.6	++++	+++++
7.1	++++	+++++
7.5	++++	+++++
8.2	++++	+++++
8.6	++++	+++++
8.9	+++	+++
9.3	++	++

– no growth
+ very faint growth
++ light growth
+++ moderate growth
++++ heavy growth
+++++ very heavy growth and the accumulation of bacteria at bottom of the streak

potentiometer to determine the reactions obtained. Bacteria from two-day-old cultures were transferred to the media and readings were made after 5 days at room temperature (70° F.). Five tubes were used in each series, and the entire experiment was run in triplicate. The results of the 3 replications were so consistent that the data from the third test (Table 4) may be taken as representative of the entire experiment.

It is obvious that *Phytomonas solanacearum* grows, at least in vitro, over a wide range of H-ion concentrations. Almost no growth occurred below pH 5 and none below pH 4.6. It made excellent growth when the pH was between 6.0 and 8.6 and developed when the pH was as high as 9.3. Its growth was not tested on media more alkaline than pH 9.3.

The cultures on media of pH 4.1 and 4.6 were transferred after 10 days to potato-dextrose agar of pH 6.8. In some cases the bacteria that had been held at pH 4.6 resumed growth on the neutral medium, indicating that not all of them had been killed by the acid medium. None of the bacteria that had been at pH 4.1 resumed growth, indicating that all of them had been killed by the acid in the medium. These results are remarkably similar to those obtained by Eddins (3), working with strains of the organism isolated from potatoes in Florida.

DISCUSSION

From the data presented in this paper it is obvious that tomato seedlings from fields in which bacterial wilt is known to be present cannot be regarded as safe for use no matter how vigorous and free from disease they may appear to be. While it may be true that infections on seedlings are severe only in localized areas in a field and, while it is not likely that a major portion of the apparently healthy plants from such field will later succumb to the disease, there is no way of knowing which plants are infected and which are not. If plants with incipient infections die soon after planting, they cannot be successfully replaced, since a healthy plant set in the space formerly occupied by a plant that died with bacterial wilt will usually contract the disease (6, 13). Thus the losses from bacterial wilt are likely to be complete and irreparable. It is necessary, therefore, to guard against setting infected plants. Since *Phytomonas solanacearum* can live over winter in the soil as far north as New Brunswick, New Jersey, it is doubly important to guard against the introduction of this organism into fields where it has not previously been known to occur.

The delay in the appearance of bacterial wilt until mid-season, after plants have grown vigorously for several weeks, may result from low soil temperatures during extended periods of cloudy weather or from earlier periods of prolonged drouth during which the soil remained very dry for a considerable period of time. By mid-season the soil temperature will have risen to 70° F. or higher, even in extended periods of cloudy weather. A few days of rainy weather would then provide the requirements for the development of the disease. It is not inconceivable that plants with incipient infections by *Phytomonas solanacearum* might remain apparently free from disease throughout the season and produce profitable crops of fruit if the proper sequence of climatic conditions prevails.

Phytomonas solanacearum usually is spread extensively in the field through a transfer of infested soil or infected plant parts by cultivating tools or by the washing caused by heavy rains. Since the rate of spread due to ordinary cultivating operations appears to be very slow, there seems to be little danger of a rapid or general spread of the disease in the field by this means.

Aside from securing disease-free plants and rotation of the crops there appears to be little that the northern grower can do to prevent or decrease the losses due to bacterial wilt. Any fertilizer which will produce vigorous,

profitable tomato plants will also provide plants in which the bacteria can thrive. Tomato plants grow best in soils having a reaction of pH 6.5 to 6.8, which is also very favorable for the development of bacterial wilt. Crop rotation, however, probably will rid the soil of the organism or at least will reduce the damage from it if non-susceptible crops are grown for several years.

The problem of the southern plant grower is more vexing. The presence of local areas of infestation in a field does not necessarily mean that the entire field is infested, but there is no way of determining the extent of the infested areas in the few days between the discovery of the disease and the time when the plants must be shipped. Since this is true the only safe course for the inspectors or buyers to follow is to reject the entire field. Should cool weather prevail throughout the brief growing period it would be impossible for the inspectors or the buyers to detect the presence of infection before the plants were shipped, and even the northern growers might not suspect presence of infection until after the plants had been set and perhaps grown for several weeks in the north.

Like the northern grower, the southern plant grower is unable to control the disease by prophylactic measures or by the use of special types of fertilizer. The margin of profit on his product per acre is so low that the application of large quantities of sulphur and lime to modify the pH of the soil sufficiently to kill the bacteria would not be profitable. Aside from crop rotation, which can reduce the incidence of bacterial wilt but will not prevent it, the only preventive practice that can be followed is a careful selection of land on which to grow tomato plants. All low, poorly-drained soils should be avoided. Fields in which the disease is known to occur should not be used. Only well-drained fields in which the disease has not been known to occur should be used, and in these fields plantings should not be made in low spots or near streams or ponds.

SUMMARY

It often is not possible to distinguish non-infected tomato plants from those having incipient *Phytomonas solanacearum* infection until they are grown in moist, warm soil.

The bacterial wilt organism can live over winter in soils at least as far north as central New Jersey.

Apparently *P. solanacearum* in the soil does not spread readily except when infested soil is moved by cultivating tools or by floodwater.

Infections may occur at soil temperatures as low as 55° F., but symptoms of bacterial wilt ordinarily do not become apparent at temperatures of 55° to 60° F. From 70° to as high as 110° F. soil temperatures, the rate of development of the disease increases with an increase in temperature.

A constant, but not necessarily a great, supply of soil moisture appears to be essential for the growth of *P. solanacearum*.

The organism is able to grow over a wide range of hydrogen-ion concen-

tration. It is killed in potato-dextrose agar cultures at pH 4, makes very little growth below pH 5, and grows best between pH 6 and pH 8.

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SOME FACTORS INFLUENCING THE SOLUBILITY OF CUPROUS OXIDE IN RELATION TO ITS TOXICITY AS A FUNGICIDE[1]

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Copper has long been recognized as one of the most effective fungicides, and in some form it has found extensive use in the control of fungus diseases of plants. Copper sulphate, partly because of its high solubility in water, was for many years the compound most frequently used. In 1919 Darnell-Smith and Ross (2) advocated the use of copper carbonate dust as a substitute for copper sulphate in treating wheat for the control of bunt. Copper carbonate, although highly insoluble in water, proved to be an effective fungicide against bunt and was much less injurious to the seed than copper sulphate. This work focussed the attention of plant pathologists on the possible fungicidal value of other copper compounds of low solubility. In recent years several so-called "insoluble" copper compounds have been found useful as fungicides.

One of the more recently exploited "insoluble" copper compounds is cuprous oxide, which was demonstrated by Horsfall (5) and others, to have considerable value as a seed protectant in the control of pre-emergency damping-off. Although highly insoluble in pure water, cuprous oxide is sufficiently soluble in moist soil to have considerable fungicidal value. Its low solubility also makes it relatively less injurious to most seeds than the more soluble copper salts. Despite this fact it has proved toxic to some seeds, and is more toxic under some conditions than others. Although its variable toxicity to seeds has limited the value of cuprous oxide as a seed protectant, it is still a promising fungicide for use as a seed protectant and for spraying and dusting.

Since the variability of cuprous oxide in both fungicidal value and in seed injury appeared to be associated with its degree of solubility under different conditions, it seemed desirable to know more about the factors influencing solubility. A review of the literature has failed to reveal any appreciable information bearing directly on the subject. Most of the work with cuprous oxide as a fungicide has been highly empirical with little attention being given to the problem of solubility in the soil in contact with germinating seeds or in close proximity to other plant tissues.

The literature is replete with references to the mechanism of the liberation of copper from a number of "insoluble" copper compounds, especially with respect to the copper in Bordeaux mixture. No attempt will be made to review all of these. Three hypotheses for the liberation of copper are quoted by McCallan (6) from the statement by Barker and Gimingham (1).

1. The copper is brought into solution by atmospheric agents—more espe-

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cially by the action of carbon dioxide of the air, *i.e.*, a purely chemical explanation.

2. The leaves, on which the mixture is sprayed, exert a solvent action on the compounds, *i.e.*, an action of the host plant.

3. The fungus itself by its excretions is responsible for the production of the soluble copper by which it is finally poisoned.

McCallan and Wilcoxon (7) showed that the salts of hydroxy acids, such as malate and perhaps others, as well as of amino acids present in the spore excretions, act on Bordeaux mixture to form soluble toxic copper hydroxy and copper amino salts. While the foregoing hypotheses were intended especially to apply to Bordeaux mixture, the same might be applied with appropriate modifications to cuprous oxide when used as a dust in seed treatment. Thus, the carbon dioxide of the soil atmosphere might exert a dissolving action, or the roots of the plant could give off products with a solubilizing effect, and lastly the fungus, in intimate contact with the dust-covered seed, might excrete a solvent for the liberation of sufficient copper by which the fungus is poisoned or inhibited.

The explanation of the mechanism of solution of cuprous oxide when used in seed treatment is made more complicated by the fact that the compound comes in contact with the various constituents of the soil, which in itself is a heterogeneous mixture, and varies in composition from point to point and from soil to soil. Thus the chemical agents exerting an important solvent action at one place might be relatively unimportant at another.

In the course of certain work concerned with another phase of this problem it was observed that a sterile neutral medium containing peptone and yellow cuprous oxide became intensely blue after standing for a short time in the incubator. Since the effect was so striking, and as it offered a clue to the mechanism of solution of cuprous oxide and a plausible explanation for the immediate toxic effects that the compound shows as a fungicide in preventing damping-off, this phenomenon was further investigated. Particular emphasis was placed upon the importance of the nitrogenous products of protein decomposition in the solution of yellow cuprous oxide when used in seed treatment.

ANALYTICAL METHODS

Tests for the solubility of yellow cuprous oxide were made using water that had been doubly distilled from all-glass equipment. One hundred ml. of this water was put into a Pyrex flask together with 2 g. of yellow cuprous oxide, placed on a shaking machine, and agitated for 10 minutes each hour for one week. The oxide was then allowed to settle, whereupon the supernatant liquid was centrifuged 3 times for 15 minutes each at 3000 r.p.m. This triply centrifuged water was tested (method 2) and found to contain from 0.6 to 0.8 p.p.m. of copper. When ordinary laboratory distilled water was used and treated in the same way, as much as 1 to 2 p.p.m. of copper was found present. Methods used for the estimation of copper:

1. Whenever the amount of copper in solution was between 100 and 800 p.p.m. the solutions were analyzed directly, but if they exceeded the upper-limit they were diluted so as to fall within this range. Four ml. of the copper solution was mixed with 1 ml. of concentrated ammonium hydroxide. The intensity of color of the resulting blue solution was determined with a Fischer Electrophotometer, using the A. filter. Reference of the reading to a standard curve gave the amount of copper in solution. The solutions used in constructing the standard curve were prepared by dissolving weighed amounts of pure copper sulphate ($CuSO_4 \cdot 5H_2O$) in water containing 1.0 per cent and 0.1 per cent of glycine.

2. The principle of the Biazzo method as modified by Elvehjem and Lindow (3) was employed for solutions containing less than 100 p.p.m. of copper. The neutral solution of copper was diluted so as to contain from 2 to 20 p.p.m. of copper. Ten ml. of this solution was placed in test tubes of uniform diameter. The following reagents were added, shaking after each addition: 1 ml. of glacial acetic acid; 1 ml. of 10 per cent potassium thiocyanate solution; 5 drops of freshly distilled pyridine, and 5 ml. of chloroform. The tubes were allowed to stand until the chloroform layer became clear. The yellow color of the chloroform layer was compared with that obtained in a set of standard tubes prepared in the same way. When the amount of copper was less than 2 p.p.m. the color was determined with a Keufel and Esser color analyzer using the wave length 450 m/w to obtain the readings. The color was compared with that of a standard copper solution similarly treated.

3. Qualitative tests for copper were made by adding 3 to 5 drops of a 5 per cent solution of a-benzoin oxime in 95 per cent alcohol to 5 ml. of the sample solution. The formation of a yellow color showed the presence of copper.

Influence of Certain Nitrogenous Compounds on Solubility of Cuprous Oxide

In order to determine the solvent action of nitrogenous substances on cuprous oxide gelatin, peptone, glycine, aspartic acid, asparagine, arginine, and cystine were tested. The materials were dissolved in distilled water in the concentrations indicated in table 1. An excess of yellow cuprous oxide was added to the flasks. These were then stoppered and placed on a shaking machine, which agitated the liquids for 10 minutes every hour during 7 days at a temperature of 25° C. The amounts of copper brought into solution by these materials are shown in table 1.

The data show that the hydrolyzed protein and pure amino acids, which were soluble in water, dissolved the cuprous oxide readily, and that this was accomplished by very dilute solutions of these chemicals. By way of contrast, the relatively unhydrolyzed protein gelatin, when used in the same concentrations, produced comparatively little effect.

In order to determine the effect of the products of bacterial decomposition of gelatin the following experiment was performed. Coarse white

TABLE 1.—*The solvent action of certain protein derivatives on cuprous oxide*

Material	Concentration	Copper in solution
	Grams per 100 cc.	*p.p.m.*
Blank	.....	0.6 to 0.8
Gelatin	0.01	2
"	0.10	10
"	1.00	125
Peptone	0.01	2
"	0.10	100
"	1.00	925
Glycine	0.001	7
"	0.01	50
"	0.10	550
"	1.00	2200
Aspartic acid	0.10	370
Asparagine	0.10	220
Arginine	0.10	185
Cystine	Saturated sol'n.	45

sand was mixed with two per cent of gelatin. Fifteen grams of this mixture was placed in a 250-ml. flask and sterilized. Four ml. of a water suspension of bacteria was mixed with the sand, and the cultures were incubated at 27° C. for 1 week. Then 0.1 g. of yellow cuprous oxide was added to each flask and mixed with the sand. This was allowed to stand for 1 day at 27° C. after which 5 ml. of water was added and the whole allowed to stand with frequent shaking for one hour. The liquid was poured from the sand and centrifuged 3 times for 15 minutes each at 3000 r.p.m. The clear supernatant liquid was tested qualitatively for copper, using a 5 per cent alcoholic solution of a-benzoin oxime as a test reagent. The results are given in table 2. In those flasks inoculated with proteolytic bacteria a greater amount of copper was brought into solution than in those inoculated with the non-proteolytic *Escherichia coli*, or in the control flasks.

Further evidence that the products resulting from the bacterial decomposition of proteins are factors in the solution of yellow cuprous oxide, was obtained by placing cubes of hard-boiled egg white weighing 1 g. in 10 ml. of sterile distilled water in flasks inoculated with a water suspension of bacteria and incubated for 3 days at 37° C. After this, the solutions were filtered through paper and yellow cuprous oxide added to the filtrate in a flask. A drop of toluene was added to each flask to prevent further growth

TABLE 2.—*The relative solvent action of the products of bacterial decomposition of gelatin*

Bacteria used	Solvent index[a]
None	1
Escherichia coli	1
Bacillus mesentericus	6
Bacillus subtilis	5
Proteus vulgaris	3
Bacteria from garden soil	4

[a] Based on the relative intensity of the color produced by the a-benzoin-oxime test.

of the organisms. After standing for 24 hours at 25° C. the solutions were cleared by centrifuging them 3 times for 15 minutes each at 3000 r.p.m. The clear solutions were tested for copper.

Eight p.p.m. of copper were found in the solution containing egg white alone. When the non-proteolytic bacterium *Escherichia coli* was added, only a slight increase of copper in solution was noted, but when proteolytic species were used the amount of copper was increased as follows: *Proteus vulgaris,* 380 p.p.m., and *Bacillus subtilis,* 670 p.p.m.

The results of this experiment show again that the products of protein decomposition are active agents in dissolving cuprous oxide. A method employing an entirely different principle demonstrated the solubility of cuprous oxide and a probable mechanism by which copper becomes effective as a fungicide when employed in seed treatment. A 4 per cent solution of the best grade of agar-agar was drawn into a glass tube with an internal diameter of 0.25 in. and allowed to solidify. The cylinder of agar was pushed from the tube into a beaker of distilled water, in which the agar was dialyzed for 24 hours with frequent changes of the water. Thereafter, the

TABLE 3.—*The solubility of cuprous oxide in several substrates as indicated by the absorption of copper by agar cylinders*

Substrate	Solvent index[a]
Control agar cylinder	0
Water saturated with Cu_2O. Excess of Cu_2O on bottom of flask	1
White sand	2
Potting soil	3
Sand moistened with 0.1% glycine water	4
Sand moistened with 1.0% glycine water	6

[a] Based on relative intensity of the color produced by the a-benzoin-oxime test.

cylinder was cut into 1-in.-long sections, the free water removed between pieces of filter paper, rolled in finely powdered dry copper oxide and then imbedded in sand or other test material, moistened and allowed to stand for 48 hours. The cylinders were then removed, washed in distilled water to remove sand, dirt, and excess copper oxide, dried between filter paper, and split lengthwise, thus giving 2 sections, with a broad surface throughout the length of the cylinder. The sections were flooded with a 5 per cent alcoholic solution of a-benzoin oxime, whereupon a greenish yellow coloration appeared, the intensity of which indicated the amount of copper absorbed by the cylinder. The data show that the composition of the soil and soil-water affect the liberation of copper (Table 3). Only a small amount of copper is put into solution by distilled water itself, however, a greater amount is liberated when organic matter of nitrogenous composition is present. This is especially true if the nitrogenous organic matter is an amino acid.

In order for a fungicide to be effective in preventing damping-off, it must go into solution rapidly enough to affect the pathogen before it has time to attack the host plant. In the case of rapidly germinating seeds, copper must be liberated shortly after the seeds have been planted, for the protec-

tive influence of the fungicide must exert itself as soon as the seed begins to sprout. In the short time required between planting and germination, some mechanism other than mere solution by water alone must be active to liberate sufficient copper.

Since the products of protein hydrolysis dissolved cuprous oxide, the following experiment was conducted to determine the speed with which the copper was released. One-half gram of cuprous oxide was added to 500 ml. of 0.1 and 0.01 per cent solutions of glycine at 25° C. and the mixture shaken frequently during the test period. At specified times samples were removed, clarified by centrifuging, and the amount of copper in solution determined. The results are given in table 4 from which it is evident that large amounts of copper are put into solution within a period of time, sufficiently short to make the toxic element available in effective concentration around the seed upon its emergence.

TABLE 4.—*The rate of solution of cuprous oxide in glycine solutions*

0.1 per cent glycine		0.01 per cent glycine	
Time of action	Copper	Time of action	Copper
hr.	*p.p.m.*	*hr.*	*p.p.m.*
0	0	0	0
½	75	½	15
3	105	1½	25
17	210	3½	50
24	280	5	55
		24	75

The results of these experiments show that the nitrogenous products of protein decomposition are active in the rapid solution of cuprous oxide. It is not suggested that this is the only or main mechanism of solution, but it does seem possible that this factor may play an important role in the action of cuprous oxide in controlling damping-off. The oxide-coated seeds lie for some time in moistened soil. The latter contains a mixture of protein in various stages of decomposition. These highly active reagents quickly liberate sufficient copper to protect the seedlings from attack by the fungus. It is also probable that this solvent action, by releasing too much copper, may account for the seed injury sometimes obtained with copper oxide.

The Toxicity of Copper Brought into Solution by Certain Nitrogenous Compounds

Increased solubility does not necessarily imply correspondingly greater toxicity. Heuberger and Horsfall (4) have shown that materials containing considerable amounts of protein, such as derris powder, pyrethrum powder, soya flour, and alfalfa meal, reduced the fungicidal value of copper compounds. These authors suggested that this action is most likely brought about by a reaction between the toxic copper and the protein, which reduces the amount of the toxic copper available to the spores. It seemed desirable,

therefore, to investigate the toxicity of the copper solution resulting from the action of these nitrogenous substances on cuprous oxide.

Methods and Materials. A master copper solution was prepared by dissolving a weighed amount of copper sulphate ($CuSO_4 \cdot 5H_2O$) in water containing the desired concentration of glycine or other test substances. This solution was neutralized to a pH of 6.2. Another stock solution was prepared by dissolving a weighed amount of yellow cuprous oxide in water containing either glycine or peptone and neutralizing this to a pH of 6.2. Dilutions of the master solutions (giving the desired concentration of copper), were prepared as required. Subsequent experiments showed that the copper was of the same toxicity from either master solution, indicating that

TABLE 5.—*The effect of different amounts of gelatin, peptone, and glycine on the toxicity of copper solutions as indicated by the relative growth of Pythium*

Composition of test medium	Copper	Relative growth of *Pythium*[a]
	p.p.m.	
Basal medium in water	0.0	4
" " " "	0.3	4
" " " "	0.5	–
" " " " + 0.1% gelatin	1.0	4
" " " " " " "	2.0	–
" " " " " " peptone	5.0	4
" " " " " " "	7.0	–
" " " " " " glycine	7.0	4
" " " " " " "	10.0	2
" " " " " " "	12.0	–
" " " " " " 1.0% gelatin	5.0	4
" " " " " " "	7.0	–
" " " " " " peptone	60.0	4
" " " " " " "	80.0	2
" " " " " " "	100.0	–
" " " " " " glycine	200.0	4
" " " " " " "	225.0	3
" " " " " " "	250.0	–

[a] 4 = Good growth.
– = No growth.

toxicity is a function of the copper ion rather than the copper compound from which it is obtained.

The basic nutrient consisted of a solution prepared by adding 1 g. of potato-dextrose agar (Difco) and 0.1 g. of peptone to 20 ml. of distilled water. This was allowed to stand with frequent shaking for 2 hours at room temperature, then filtered. The solution was sterilized and 0.2 ml. of it was added to 10 ml. of the sterile copper-containing test solution. This procedure was employed in order to reduce decomposition of the nutrients to a minimum.

The test organism was a laboratory strain of *Pythium debaryanum,* which caused active damping-off of spinach and other seedlings. The fungus was grown in the basic medium for 5 days. Small pieces of mycelium were added to 10 ml. of test solution contained in a 250-ml. Erlenmeyer flask.

The flasks were incubated at 27° C. for 5 to 7 days and then examined for the amount of growth.

Experimental Results. The effect of gelatin, peptone, and glycine upon the toxicity of copper to *Pythium* is recorded in tables 5 and 6. In the experiment reported in table 5 the growth of *Pythium* was estimated. In the one reported in table 6 growth was measured by determining the dry weight of mycelium formed. These results show that the threshold of toxicity was between 0.3 and 0.5 p.p.m. in a solution containing only the basic nutrients. Upon the addition of gelatin, peptone, and glycine, the tolerance for copper increased. The reduction of toxicity varied with the kind and amount of

TABLE 6.—*The effect of different amounts of gelatin, peptone, and glycine on the toxicity of copper solutions as indicated by the dry weight of mycelium formed by Pythium*

Composition of test medium	Copper	Dry wt. of mycelium
	p.p.m.	*mg.*
Basal medium in water	0.0	6.5
" " " "	0.3	5.1
" " " " + 0.1% gelatin	0.0	15.5
" " " " " " "	0.5	7.8
" " " " " " "	1.0	9.1
" " " " " 1.0% "	0.0	15.1
" " " " " " "	3.0	13.5
" " " " " " "	5.0	14.1
" " " " " 0.1% peptone	0.0	13.1
" " " " " " "	1.0	9.9
" " " " " " "	3.0	7.7
" " " " " 1.0% "	0.0	26.0
" " " " " " "	40.0	18.3
" " " " " " "	60.0	20.1
" " " " " 0.1% glycine	0.0	11.8
" " " " " " "	3.0	7.5
" " " " " " "	5.0	6.5
" " " " " 1.0% "	0.0	11.1
" " " " " " "	150.0	5.9
" " " " " " "	200.0	6.9

nitrogenous substance added to the medium. The depression of the toxic action was least pronounced in the case of gelatin, a slightly hydrolyzed protein, and progressively more in the case of peptone and glycine, both of which result from the extensive hydrolysis of proteins. Furthermore, the neutralizing influence increased with an increase in the concentration of test material.

An estimate of the toxicity-depressing effect can be obtained from table 7. Whereas the threshold of toxicity in the basal medium alone was between 0.3 and 0.5 p.p.m. of copper, the addition of 0.5 per cent of glycine permitted the growth of the fungus in only slightly diminished amounts until a concentration of 100 to 110 p.p.m. was reached, after which growth ceased.

In order to determine the amount of glycine necessary to neutralize the toxicity of a given amount of copper an experiment was conducted wherein the amount of copper in solution was kept constant and the amount of

TABLE 7.—*The effect of 0.5 per cent glycine on the toxicity of different concentrations of copper as indicated by the dry weight of mycelium formed by Pythium*

Composition of test medium	Copper	Dry wt. of mycelium[a]
	p.p.m.	*mg.*
Basal medium	0.0	7.0
" "	0.3	7.2
" "	0.5	1.2
" " + 0.5% glycine	0.0	10.8
" " " " "	60.0	9.3
" " " " "	80.0	8.4
" " " " "	100.0	9.5
" " " " "	110.0	0.7

[a] The weight of the combined mycelium from three replicate flasks.

glycine progressively increased. The results are given in table 8. The data show that from 0.5 to 0.6 per cent of glycine were required to overcome the toxicity of 100 p.p.m. of copper.

It has been shown that dilute solutions of glycine dissolved many times as much cuprous oxide as was dissolved by distilled water. The question arises as to what extent a given amount of glycine would reduce the toxicity of the copper that it put into solution. The data in table 9 show the relationship between the dissolving power of glycine and the toxicity of the copper-glycine complex. From the table it may be seen that the copper-glycine complex is toxic in concentrations far below the total amount of copper that is put into solution by glycine. In order to be toxic approximately 500 times as much copper is required when 1 per cent of glycine is present as is necessary in the basal medium alone.

These results confirm the conclusions of Heuberger and Horsfall (4) that nitrogenous compounds may reduce the fungicidal value of copper compounds. This is true, however, only when an excess of the materials is present. Smaller quantities may actually increase the toxicity by increasing the solubility of the copper compound. It is not known definitely in what way excess nitrogenous compounds decrease the toxicity of the copper,

TABLE 8.—*The amount of glycine required to neutralize the toxicity of a 100 p.p.m. solution of copper as indicated by the relative growth of Pythium*

Glycine	Copper	Relative growth[a]
%	*p.p.m.*	
0.0	0.0	4
0.1	100.0	–
0.2	"	–
0.3	"	–
0.4	"	–
0.5	"	–
0.6	"	3
0.7	"	4

[a] 4 = Good growth.
– = No growth.

but it is probably due to reduced ionization of new compounds formed by the interaction of the two materials.

Nikitin and Anderson (8) studied the effect of various protein-containing supplements on the adherence and activation of fixed copper fungicides. Their results showed that the magnitude and tenacity of the spray residue are considerably increased by supplements containing proteins. Furthermore, these supplements exert a dissolving action on fixed copper fungicides. However, no studies were reported on the toxicity of the copper put into solution by the proteins. Since these findings (with respect to the solution of copper) were in line with those of the writers when using cuprous oxide, a study was made of the dissolving effect of soya-bean flour under specific conditions and the toxicity of the resulting solution.

One gram of finely ground soya-bean flour was mixed with 100 ml. of distilled water. This was allowed to stand in the ice box with frequent

TABLE 9.—*The relationship between the dissolving power of glycine and the toxicity of the copper-glycine complex*

Concentration of glycine	Copper dissolved	Threshold of toxicity[a]
%	*p.p.m.*	Cu *p.p.m.*
0.000	0.8	0.3 to 0.5
0.001	7.0	0.3 to 0.5
0.010	50.0	0.5 to 0.6
0.100	550.0	0.8 to 1.0
0.500	1800.0	90 to 100
1.000	3200.0	225 to 250

[a] "Threshold of toxicity" means the smallest amount of copper required to inhibit the growth of *Pythium*.

shaking for 48 hours. Thereafter, the liquid was filtered through paper and further clarified by centrifuging. An excess of washed Bordeaux-mixture precipitate was added to the soya-bean extract and allowed to stand with frequent shaking at 20° C. for 48 hours. The mixture was clarified by centrifuging and filtering. A control, using water and Bordeaux mixture, was treated in the same way. The resulting solutions were tested for copper and for their toxicity toward *Pythium debaryanum*.

Copper was not detected in the water control, while the soya-bean-extract sample contained 220 p.p.m. of copper. The control solution was not toxic, whereas the soya-bean-extract preparation was completely toxic in a dilution of 1:400, but permitted growth in a dilution of 1:450. The amount of copper in the highest dilution of soya-bean-copper solution was approximately 0.5 p.p.m., thereby indicating that the toxicity of this soluble copper complex is of essentially the same magnitude as that of the copper from inorganic sources, such as copper sulphate.

Experiments were conducted to determine the effect of soya-bean flour on the solution of yellow cuprous oxide and to test the toxicity of the copper solutions so obtained. A 1 per cent suspension of soya-bean meal was dispersed in 100 ml. of water. An excess of yellow cuprous oxide was added

to the mixture, which was allowed to stand, with frequent shaking, for 4 days at 10° C. The resulting solution was clarified by filtering and centrifuging. Analysis of this solution showed that it contained 125 p.p.m. of copper. *Pythium debaryanum* did not grow in this solution when tested in the usual way. Furthermore, the solution, upon dilution with distilled water, prevented growth of the fungus at a concentration of 0.6 p.p.m. of copper. When fresh soybean meal was added to the clarified solution a reduction in toxicity occurred. The threshold of toxicity rose to 2 to 3 p.p.m. when 0.1 per cent of meal was added, and complete loss of toxicity resulted when 1 per cent was used.

Thus it was shown that soya-bean flour is capable of activating the copper of cuprous oxide in far greater quantities than the minimum necessary for a toxic concentration. Some of the liberated copper is unquestionably bound by the soya-bean flour, however, the amount liberated is greater than the minimum amount necessary for the inhibition of growth of the fungus, so long as an excess of active soya-bean is not present. The addition of an excess of soya-bean flour to a solution containing copper reduces its toxicity. This fact should be kept in mind when using protein-containing supplements with copper fungicides, as suggested by Nikitin and Anderson (8).

SUMMARY

A study has been made of certain factors influencing the solubility and toxicity of cuprous oxide using *Pythium debaryanum* as a test organism.

Growth of *P. debaryanum* in a standard liquid medium was inhibited by cuprous oxide at a concentration of 0.3 to 0.5 p.p.m. of copper in solution. As much as 0.6 to 0.8 p.p.m. of copper was brought into solution by prolonged action of doubly distilled water. Ordinary laboratory distilled water dissolved from 1 to 2 p.p.m.

The solubility of cuprous oxide is greatly increased by glycine and other nitrogenous products of protein decomposition. As much as 2200 p.p.m. was liberated by the addition of 1 per cent glycine. The copper liberated by these nitrogenous compounds is equally as toxic as the copper dissolved in distilled water provided no excess of nitrogenous compounds is present. However, the threshold of toxicity in p.p.m. is greatly increased when an excess of these compounds is present, 1 per cent glycine raising the toxicity threshold from 0.3 p.p.m. to 225 p.p.m.

Soya-bean flour increased the solubility of cuprous oxide, a 1 per cent suspension dissolving 125 p.p.m. The copper dissolved by soya-bean flour inhibited growth of *P. debaryanum* when diluted to 0.6 p.p.m. but when 0.1 per cent of soybean meal was added to the diluted solution the threshold of toxicity was increased to 2 to 3 p.p.m. and toxicity was completely lost by the addition of 1 per cent soya-bean flour.

Because nitrogenous products of bacterial decomposition are known to be present in varying amounts in arable soils, it is very probable that they influence the solubility of cuprous oxide used as a seed protectant. They

may account for some of the variability in seed protection and seed injury experienced with cuprous oxide.

In as much as the influence of nitrogenous compounds on the toxicity of copper fungicides depends, not only upon the nature of the compound, but also on the ratio between the two substances, caution must be used in evaluating supplements containing proteins. Excess of protein supplements may decrease the toxicity of copper fungicides, while smaller amounts may, by increasing the solubility of copper compounds, increase their fungicidal value.

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VARIATION IN SYMPTOMS PRODUCED BY ISOLATES OF PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

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INTRODUCTION

In greenhouse halo-blight-resistance tests, inoculated bean leaves were observed on which necrotic halo-less lesions appeared, occasionally in addition to lesions with characteristic halos. Since the inoculations were carried out with an intentional mixture of several isolates and, since the 2 types of lesions sometimes appeared side by side on the same leaf, it seemed probable that the atypical ones might have resulted from infection with certain strains of the bacterium rather than because of temperature or varietal reaction.

It has been noted by several investigators that symptom variations in halo blight occur. Burkholder (1) noted that halos are never produced around the small, necrotic leaf lesions when infection takes place in the hot days of July or August. Goss (3) demonstrated that only halo-less lesions appeared at 28° and 32° C. on leaves of Red Kidney and U.S. No. 5 Refugee, whereas typical halos surrounded lesions on leaves of plants of these two varieties held at 16°, 22°, and 24° C. More recently (4) it was shown that in certain varieties, Mexican Red, Schwert, and others, only small, necrotic halo-less lesions were produced under a wide range of temperatures (16°–28°) and at all ages of growth, whereas Red Kidney and Bountiful plants inoculated at the same time with the same inoculum produced lesions with characteristic halos at 16° and 22°.

In preliminary tests with 6 isolates it was observed that at least 2 isolates regularly produced some leaf lesions that lacked the characteristic halos. Further tests with isolates from several parts of the United States, as well as other isolates from Nebraska, showed conclusively that in addition to the variation in halo production a rather wide range of symptoms is produced by isolates of *Phytomonas medicaginis* var. *phaseolicola* (Burkh.). A description of the range of these symptoms with various inoculation methods on Red Kidney beans and a summary of the physiological studies with these isolates on artificial media are presented in this paper.

MATERIALS AND METHODS

The 13 isolates of *Phytomonas medicaginis* var. *phaseolicola* Burkh. used in these studies were obtained from naturally infected field-grown beans from various localities including Wisconsin, Montana, Louisiana, and Nebraska. Since the isolates had been in culture on artificial media for lengths of time varying from several weeks to several years, it seemed desirable to attempt to eliminate the possible variations due to age-of-culture effect.

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Accordingly, all isolates were inoculated by needle puncture into stems of young, healthy Red Kidney plants whence they were isolated after a period of several weeks. All studies reported here were carried out with the reisolates, whose pathogenic characteristics were identical with the original isolates.

Single-cell isolations of the bacteria were not attempted. All stock cultures were obtained from isolated single colonies on plates poured with high dilutions. That striking differences were obtained by cultures from isolated single colonies indicates that similar differences might be found when working with colonies grown from single bacterial cells.

Stock cultures were maintained on bean-decoction agar consisting of the boiled decoction of 200 g. of bean leaves made up to a liter of 2 per cent agar. Physiological studies were carried out on media made up according to standards adopted by the Society of American Bacteriologists (2).

Plants were grown in composted soil in 5-inch porous clay pots in the greenhouse during autumn, winter, and spring months. Greenhouse temperatures were held at approximately 22° C.

Studies of the symptoms produced by the various isolates were made by using 4 inoculation techniques: leaf, stem, germinated seed, and pod inoculations. Although complete descriptions of these methods are presented in a previous publication (4), a brief description is given here. Leaf and pod inoculations were made by spraying plants with a bacterial suspension in water. Previous to inoculation, plants were held overnight in an incubation chamber at 24° C. maintained at a high humidity. Following the inoculation plants were held in the incubator for 6 or 7 hours before removal to a greenhouse bench. Leaf inoculations were made just as the third trifoliate leaves were unfolding, and pod inoculations were made during pod development. Stem inoculations were made by stabbing twice through a smear of bacteria placed on the bean stems about $\frac{1}{2}$ cm. below the primary leaves. The method used in making germinated seed inoculations consisted of soaking germinated seeds for 4 hours in a dilute bacterial suspension in water. Seeds were germinated in a moist rag-doll held for 60 hours at 27°–28° C.

Except in the case of pod inoculations, these pathogenicity tests were conducted by making inoculations with all isolates at the same time. The incubation chambers were too small to accommodate at one time all of the nearly mature plants used in pod inoculation; therefore, inoculations were made in 2 sections carried out on successive days.

Following the first complete series of tests, conducted with bacteria isolated from inoculated Red Kidney plant stems, all isolates were transferred to agar slants which, after a day's growth of the bacteria, were covered with sterile mineral oil (5). The 4 types of inoculation tests were repeated a number of times over a 4-year period with isolates stored under oil for periods varying from several months to several years. Such extensive tests failed to show any major changes in pathogenicity or in growth characteristics as a result of storage. In some tests isolates that had been stored under

oil were compared with the same isolates maintained by routine laboratory transfer, and no changes ascribable to either treatment were shown.

EXPERIMENTAL RESULTS

Leaf Inoculations

Healthy, young Red Kidney plants were sprayed with water suspensions of the bacteria. Five plants were used for each isolate. The first symptoms appeared within 5 days on plants inoculated with certain isolates; whereas, in other cases the first symptoms appeared 1 to 3 days later. Final readings were not made until 15 to 20 days after inoculation. Table 1 presents

TABLE 1.—*Some differential symptoms produced on Red Kidney beans by 13 isolates of Phytomonas medicaginis var. phaseolicola in four methods of inoculation. Isolates are presented in three groups, the spaces separating them on basis of pathogenicity*

Isolate number	Leaf inoculation		Stem inoculation				Germinated seed inoculation[c]	Pod inoculation
	Lesion type	Systemic infection	Stunting	Vein-clearing	Wilting	Mottling	Vein-clearing primary leaf	Lesions
2076[a]	Halo	++[b]	+++	+++	+++	0	+++	Typical
2080	Halo	++	+++	+++	+++	0	+++	Typical
2082	Mixed	0	+++	+++	+++	0	+++	Typical
2071	Mixed	0	+++	+++	+++	0	+++	Typical
2072	Mixed	0	++	++	++	0	++	Typical
2074	Mixed	0	++	++	++	0	0	Small
2077	Mixed	0	++	+	++	++	0	Typical
2078	Mixed	0	++	+	++	0	0	Typical
2081	Mixed	0	++	+	++	0	0	Typical
2073	Halo-less	0	++	+	+	++	0	Small
2079	Halo-less	0	+	0	0	++	0	Small—few
2083	Halo-less	0	+	0	0	++	0	Small—few
2075	Halo-less	0	0	0	0	0	0	Small

[a] The authors are indebted to John McLean, Colorado State College, Fort Collins, Colo.; Lee Person, Louisiana State University, Baton Rouge, La.; M. M. Afanasiev, Montana State College, Bozeman, Mont., for some of the isolates used in these tests.

[b] Symbols: +++ = severe, ++ = moderate, + = mild, 0 = did not occur.

[c] Stem cankers were produced by all isolates with germinated-seed inoculation method.

a summary of the symptoms produced by all isolates and lists them in 3 general groups or levels of pathogenicity. Large, halo lesions always appeared on plants inoculated with 4 of the isolates. Small, necrotic, halo-less lesions invariably appeared on plants inoculated with 4 other isolates. On the other hand, a mixture of halo and halo-less lesions appeared on plants inoculated with the remaining isolates. Where mixed lesion types occurred, these 2 types of lesions frequently appeared side by side on the same leaf. Figure 1, G, H, and I presents photographs of typical halo, halo-less, and mixed lesion types.

Usually, isolates producing halo or mixed lesions also produced the greatest number of lesions. An exception, however, was also observed. One

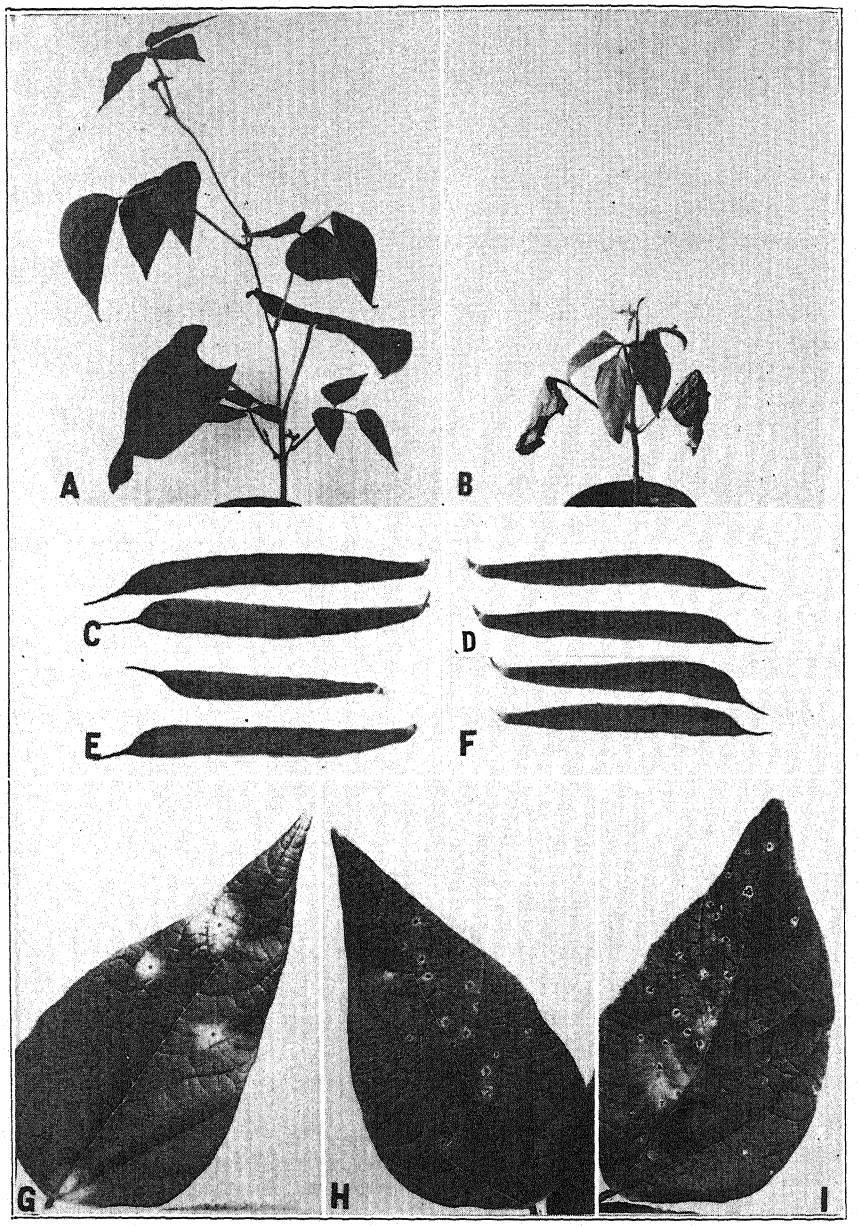


FIG. 1. Red Kidney plants, leaves and pods inoculated with various isolates of *Phytomonas medicaginis* var. *phaseolicola*. A. Eighteen days after stem inoculation with isolate 2079. Aside from a slight stunting and some faint mottling in the uppermost leaves, this plant showed no symptoms and grew as vigorously as uninoculated controls. B. Eighteen days after stem inoculation with isolate 2071. Severe stunting, wilting and vein-clearing symptoms are shown. C, D, E, and F. Pod lesions produced by spray inoculation with isolates 2076, 2079, 2074, and 2081, respectively. Pod lesions produced by isolates 2074 and 2079 were uniformly slightly smaller in size and those of 2079 were fewer in number than those produced by isolates 2076 and 2081. G, H, and I. Leaves spray-inoculated with isolates 2080, 2083, and 2077, respectively. The three leaves show (G) typical halo, (H) halo-less, and (I) both halo and halo-less lesions.

isolate producing only halo-less lesions regularly produced more lesions than any other isolate.

In some instances leaf inoculation of young, vigorously growing plants was followed by systemic infection. In all cases systemic infection was obtained only with isolates producing halo lesions. Thus the isolates varied in the number of lesions produced, in the time interval required to produce visible lesions, in the types of lesions produced, and in ability to produce systemic infection.

Stem Inoculations

Stem-puncture inoculations into young Red Kidney plants produced a wide range of symptoms. Inoculations made with a needle just below the primary leaves were usually carried out when the first trifoliate leaves were about one-half expanded. Usually each isolate was inoculated into 5 plants in each test.

Isolates producing halo or mixed type lesions on leaf inoculation, produced characteristic symptoms of the disease with stem inoculation. Four to 5 days after stem inoculation a slight wilting of the primary leaves occurred. By 6 days the first trifoliate leaflets, instead of being presented flatly, in a plane, more or less parallel to the ground line, were all pointing downward and exhibiting a slight flaccidity. This position is similar to that commonly assumed by bean leaflets at night. The reaction was especially noted in plants inoculated with some of the most virulent isolates. Six to 10 days after inoculation vein-clearing symptoms appeared. At about the same time wilting of one or more of the trifoliate leaves occurred. Within 15 to 20 days after inoculation the plants inoculated with virulent isolates were dead.

As a contrast to the extremely harmful effects just described, isolate 2075 under the same conditions produced no noticeable symptoms. Plants inoculated with this isolate could not be distinguished from healthy, uninoculated controls, except at the point of inoculation where slight discoloration and water-soaking of the nearby tissues occurred. Inoculated plants produced a set of pods which at maturity could not be distinguished from those on uninoculated controls.

Between these 2 extremes, other isolates produced some stunting, some wilting, and usually vein-clearing symptoms. However, the inoculated plants lived almost as long as control plants, which, although punctured with sterile needles, lived to complete maturity and produced a satisfactory crop of beans. In only one instance were vein-clearing symptoms produced by an isolate that produced only halo-less lesions on leaf inoculation.

Symptoms exhibited by plants inoculated by stem puncture thus fall into several groups. The first group consists of those showing marked stunting as early as 6 days after inoculation; and in these plants vein-clearing usually appeared at about the same time. A second group of isolates produced vein-clearing as early as 6 days after inoculation, but no noticeable stunting of inoculated plants occurred until several days later. In a third

group only extremely mild symptoms, such as a slight wilting of the primary leaves, or no symptoms at all, were produced. Photographs in figure 1, A, and 1, B, illustrate the range of symptoms obtained by stem inoculation on Red Kidney beans.

In some instances of stem inoculation a marked mottling appeared in the leaves near the growing point. This symptom occurred on fully grown plants that had been inoculated when young, but before any general yellowing or other similar signs of maturity appeared. Invariably the mottling symptoms occurred only with isolates that produced few or no typical symptoms of systemic infection.

Germinated Seed Inoculation

Seeds, germinated in rag-dolls at 28° C. for 66 hours, were placed in an aqueous bacterial suspension of each of the various isolates for a 4-hour period. At the end of this treatment each germinated seed was planted in soil in a porous clay pot. Usually, 10 germinated seeds were inoculated by the above method with each isolate.

All isolates produced typical water-soaked lesions on the cotyledons, and within 3 weeks every inoculated plant also showed halo-blight stem cankers. The isolates produced varying results in the development of systemic infection, as characterized by vein-clearing symptoms on the primary leaves and in the occurrence of premature death. Isolates that produced vein-clearing symptoms on the primary leaves produced death of the plants within the following 3-week period. Isolates that produced only halo-less lesions on leaf inoculation caused lesions on the cotyledons and cankers on stems with germinated seed inoculation, but did not produce vein-clearing symptoms of the primary leaves or any other symptom of systemic infection. In many instances the plants died before maturity, depending upon the severity of the stem cankers, but in other instances, in spite of stem cankers, the plants grew to maturity.

Pod Inoculations

A group of large, healthy Red Kidney plants with some of the pods on each plant nearly full-size and other pods still developing, was divided into 15 similar lots of 5 plants each. A water suspension of bacteria of each of the 13 isolates of the bacterium was sprayed on the pods of the respective lots with an atomizer. A check lot of 5 plants was sprayed with sterile water in each group of inoculations. After inoculation all plants were incubated in a moist chamber for 8 hours, after which they were placed on a greenhouse bench.

Lesions developed on pods of all lots within 4 to 8 days after inoculation. Final observations on pod infection were made 2 weeks after inoculation. Although leaf, stem, and germinated seed inoculations with the various isolates produced striking variations in symptoms, pod infection symptoms were all of the same general type, but varied in size and number of lesions. Several isolates, which were characterized by low pathogenicity in previous

comparative tests, produced lesions slightly smaller in diameter than those resulting from more virulent isolates. In general, a similar number of pods were infected, and approximately the same numbers of lesions were produced with each isolate although two isolates induced notably fewer lesions. None appeared on pods sprayed with sterile water.

Figures 1, C, D, E, and F, present photographs of pod lesions resulting from inoculation with several representative isolates.

EFFECT OF TEMPERATURE ON LESION TYPE

Because of the known effect (3) that high-temperature growing conditions have on the appearance of lesions of bean halo blight, it seemed desirable to study the effects of various temperatures on the lesions produced by several different isolates. Four isolates, 3 which produce only halo-less lesions and 1 which produces only typical halo lesions, were used in the study. Sixty young, healthy Red Kidney bean plants were divided into 4 similar lots of 15 plants each. Each lot was inoculated with a different isolate and then so distributed that the 5 plants inoculated with each isolate were held at 16, 22, and 28 degrees C. Table 2 records the results of this

TABLE 2.—*Effect of temperature on symptoms. Lesion type obtained when plant lots were leaf-inoculated with the indicated bacterial isolate and held at various temperatures*

Isolate number	Temperature °C.		
	16	22	28
2075	Halo-less	Halo-less	Halo-less
2079	Halo-less	Halo-less	Halo-less
2083	Halo-less	Halo-less	Halo-less
2076	Halo	Halo	Halo-less

test. It will be noted that the isolates producing halo-less lesions at 22° C. produced the same type of lesions at both 16° and 28°. In the case of the isolate producing typical halo lesions at ordinary temperatures, halo-less lesions resulted at 28° C. while at 16° and 22° typical halo lesions developed. This test confirms previous reports (3) to the effect that typical halo-blight isolates produce only halo-less lesions at 28° C., and it further shows that isolates causing halo-less lesions at ordinary temperatures (20–22° C.) also induce only halo-less lesions at 16 and 28°.

DIRECT INOCULATION FROM INFECTED TISSUE

In an attempt to determine whether or not bacteria from small, necrotic lesions would in turn produce only small, necrotic lesions, bacteria were transferred direct from small, necrotic or halo-less lesions to healthy, young plants. Other inoculations also were made with bacteria taken directly from typical halo lesions. Small areas of the leaf containing the lesions were cut out with sterile instruments and macerated in sterile mortars with sterile pestles. A water suspension of this material was then sprayed on leaves in

the manner described for leaf-inoculation technique. Necrotic lesions developed on leaves sprayed with bacterial suspension obtained from necrotic lesions. Halo lesions developed on leaves sprayed with a bacterial suspension of material from halo lesions. Occasionally, lesions, unlike those from which the inoculum was obtained, appeared in plants inoculated from either type of lesions. This would seem to indicate that variant strains of the halo-blight organism are continuously arising, or that mixtures were sometimes present in the lesions. Even in the latter case the presence of a mixture in the lesions would indicate that strain variations occur.

PHYSIOLOGICAL CULTURE STUDIES

The wide range of variation in symptoms obtained by leaf and stem inoculation of Red Kidney beans with the 13 halo-blight isolates suggested that such differences might be correlated with variations in physiological activity. Comparative studies on various types of media, commonly utilized for such observations, were carried out. In all experiments, observations were made on the 13 isolates of *Phytomonas medicaginis* var. *phaseolicola* together with an isolate of the bacterium causing common blight of beans, *P. phaseoli.*

The tests involved growth characteristics and rates-of-growth studies on beef extract agar streaks, beef extract broth, gelatin, plain milk, and milk with litmus and with brom-cresol-purple. Studies also were made on production of ammonia, hydrogen sulphide, starch digestion, and on the utilization of starch, dextrose, and sucrose. In all tests the various isolates of *Phytomonas medicaginis* var. *phaseolicola* showed characteristics that agreed with those described by Burkholder (1). In these tests the only variations obtained were slight differences in growth rates. These differences could not be correlated with those in pathogenicity.

DISCUSSION

The occurrence of a number of strains or variants of a given species or the variation in pathogenicity among isolates of a pathogenic species has been found the rule rather than the exception in biology. The discovery and description of such variation is now in itself of comparatively minor importance or significance. Frequently, however, a knowledge of the range of characteristics within which most members of a pathogenic species fall aids greatly in understanding more completely the organism and the disease produced by it. Such is the case in this report of the variation in symptoms produced on Red Kidney beans by various isolates of *Phytomonas medicaginis* var. *phaseolicola.*

With the discovery of physiological resistance in certain varieties to halo blight and the probable usefulness of such varieties in a breeding program, there has developed a need for a more thorough understanding of the various factors that influence the symptomatic picture of diseased plants in the field

and greenhouse. In a breeding program it is necessary to grow and inoculate large numbers of hybrid progenies. Selection of resistant plants must be made rapidly and usually without the benefit of laboratory pure-culture determinations of the causal organism. To do this, studies of the various factors responsible for symptom variations are essential.

This report now brings to three the number of factors shown to influence the symptoms of beans infected with halo blight. High temperatures were shown (3) to limit the formation of the typical halo development in susceptible varieties. Later, it was demonstrated (4) that certain varieties were physiologically resistant and manifested this resistance by the formation of small, necrotic, halo-less lesions instead of the large, characteristic halos commonly observed. This report now shows that certain isolates of the halo-blight organism may produce all halo or all non-halo or a mixture of these two types of primary lesions on susceptible varieties. In addition, the report describes systemic symptom differences that result from various isolates.

SUMMARY

Studies were made on the variations in symptoms produced on Red Kidney beans inoculated with 13 halo-blight (*Phytomonas medicaginis* var. *phaseolicola* Burkh.) isolates from naturally infected field-grown beans. Four inoculation techniques were used: leaf, stem, germinated seed, and pod. On the basis of pathogenicity exhibited in the various inoculation tests the isolates fall into 3 general groups. Four isolates were characterized by producing all halo or both halo and halo-less primary leaf lesions, typical pod lesions and marked stunting, wilting, vein-clearing symptoms and premature death with systemic infection. Another group of 4 isolates was characterized by the production of halo-less primary leaf lesions and small and reduced numbers of pod lesions. Stem inoculations with these same isolates produced little or no stunting or wilting and rarely vein-clearing symptoms or premature death. The third or intermediate group consisted of 5 isolates that usually produced mixed halo and halo-less primary leaf lesions and whose pathogenicity was intermediate between the two extreme groups just described.

In studies on the effect of temperature on primary lesions it was found that several isolates that induced only halo-less primary lesions at 22° C. also induced only halo-less lesions at 16° and 28°, as contrasted with the behavior of other isolates, which produced halo-less lesions at 28° but produced typical halo lesions at 16° and 22° C.

Water suspensions of macerated tissue from typical halo lesions when sprayed on young leaves caused typical halo lesions, whereas bacteria from halo-less lesions caused halo-less lesions. Occasionally, lesions unlike those from which the inoculum was obtained appeared in the inoculated plants.

In all physiological culture tests the various isolates showed characteristics that agreed with those described by Burkholder (1). In these tests the

only variations obtained were slight differences in growth rates, which could not be correlated with differences in pathogenicity.

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SEEDLING INVASION OF THE COVERED SMUT OF OATS

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The behavior and sexuality of the various oat smuts in culture have been subjected to considerable investigation. Many authors, Dickinson (4), Holton (5, 6), and Utter (12), have emphasized the heterosexuality of the oat smuts, and there is considerable evidence to show that monosporidial lines do not infect the host, but that fusion of the sporidia of presumably different sexes result in an infection hypha. Western (11) studied, in *Ustilago avenae,* the behavior of 2 monosporidial lines of unlike sex introduced by pressure under the glumes of oat seeds. Under these more natural conditions, there was a union of adjacent promycelial segments by means of a fusion tube, which finally gave rise to the true infection hypha. Tisdale and Tapke (8), Tapke (9), Kolk (7), and Western (10) also have described the invasion of different hosts by the promycelium directly produced from chlamydospores. Since 1936, the results of experiments designed to discover to what extent seedlings are invaded by the promycelia from the chlamydospore of the covered smut of oats have been carried on and are reported here.

EXPERIMENTAL

The covered smut *Ustilago levis* (*U. Kolleri*), race 7, and the oats, var. Monarch, which is 100 per cent susceptible, and Markton, which is completely resistant to it, were used. Kolk (7), Western (10) and Brandwein (1, 2), have proved beyond doubt that the smut penetrates resistant and susceptible coleoptiles alike. In Markton the inoculation results definitely in seedling invasion and sometimes in non-sporulating infection (Brandwein (3)).

Dehulled seeds of Monarch and Markton were dusted with dry chlamydospores and the seeds were then germinated in sand having a moisture content of 20 per cent of its water-holding capacity. This sand was contained in paper cups and maintained at 20° C. Twenty-four-hour-old seedlings also were thoroughly dusted with spores, and new sand of the proper moisture content was then replaced. The plants inoculated at the seed stage were removed after 48 hours for examination, the others inoculated at 24 hours were removed at 72 hours. Most useful preparations were made by stripping the coleoptile with forceps and examining the unstained tissue by suitable lighting under 440 and 950 (oil immersion) magnification. Successive strips from one plant permitted a fairly thorough examination of from $\frac{1}{3}$ to $\frac{1}{2}$ of the outer coleoptile tissue. The results are tabulated in table 1.

It was surprising to find that of the tremendous numbers of spores found on a seedling inoculated at 24 hours there were very few germinations—roughly 10 per cent—and few cases of what could be definitely called invasion.

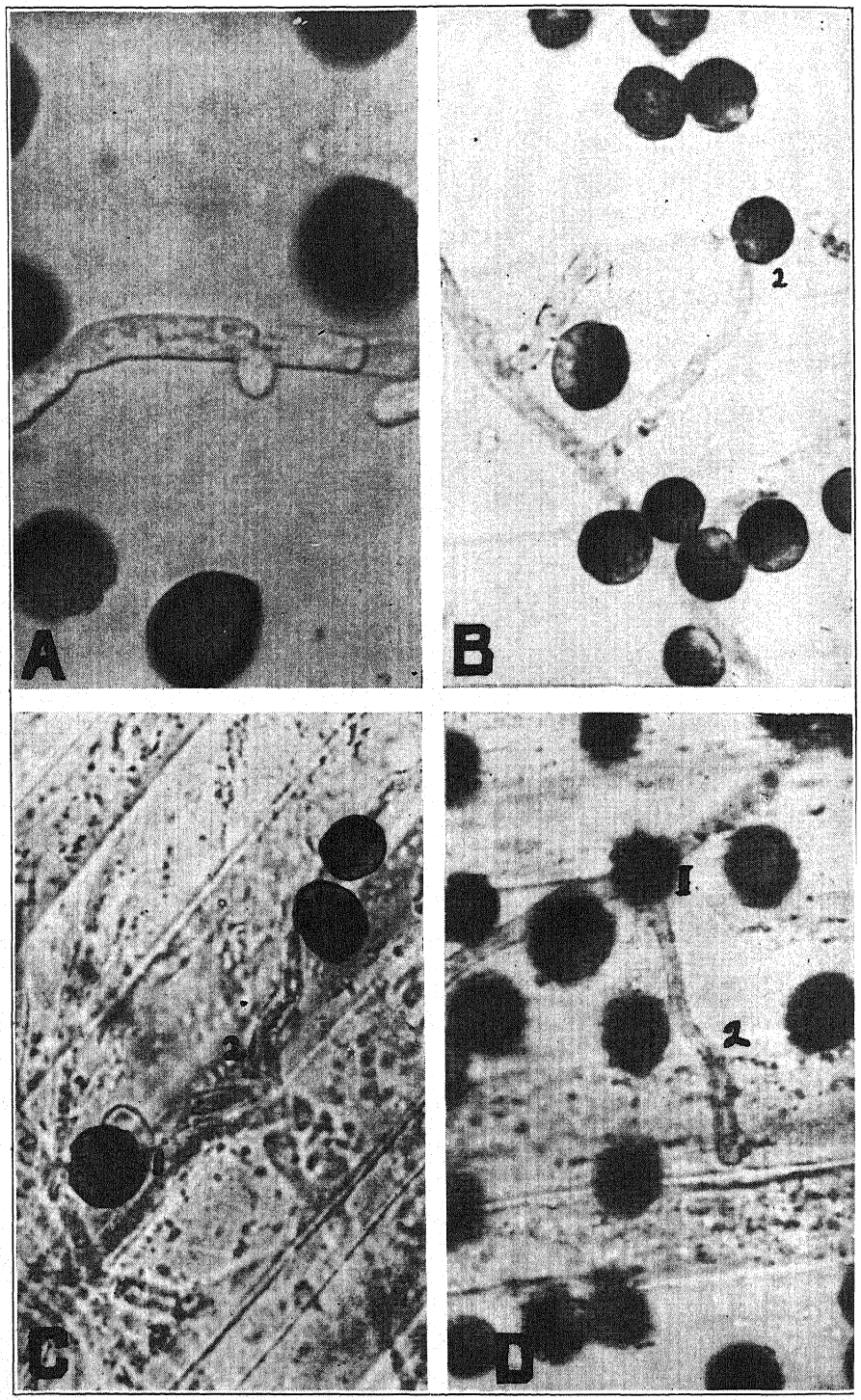


FIG. 1. Chlamydospore germination and promycelia invasion of the oat coleoptile.

Fusion of sporidia was relatively uncommon. Of the 532 cases of hyphal penetration that seemed to result from fusion, only 52 seemed to be the result of fusion of sporidia or sporidia-like structures.

Formation of sporidia (Fig. 1, A) and sporidial fusions were, however, commonly found in the mat of smut spores that sometimes was found in the sand alongside the seed.

Figure 1, B, shows a type of penetration (in Monarch) commonly found; the chlamydospore promycelium enters at 2, produces a ramifying mycelium internally which finally dips into the tissues. On the other hand, Fig. 1, C, shows 2 penetrations in Monarch from 2 promycelia, with the external and

TABLE 1.—*Spore-germination and host-invasion results obtained from oat seedlings grown from dehulled seeds of Markton and Monarch oats that had been dusted with chlamydospores of Ustilago levis*

No. seedlings examined	Host	Age of seedling (hours)	No. of invasions	Invasions directly from chlamydospore	Doubtful[a] or from fused hyphae
47	Monarch	48	201	71	130
46	"	72	407	219	188
23	Markton	48	130	32	98
27	"	72	184	68	116
143			922	390	532

[a] In 286 cases, it could not be readily determined, because of ramification and branching of the mycelium, whether penetration was that of a hypha formed by fusion of elongated promycelial segments or that of the promycelium.

internal mycelia ramifying and bunching in and on the coleoptile. In this type it was difficult to determine whether the penetration was directly from a chlamydospore or from fusion at 1 and 2 (Fig. 1, C). Fig. 1, D, shows the internal mycelium from the spore 1 in a Markton coleoptile. It is interesting to note a swelling of the cell wall just in front of the penetration hypha, which had entered at 2. Western (11) has described similar pads of cellulose. The writer has observed 3 more cases similar to this in living material. It is obvious that such favorable material would be difficult to obtain. Figures 1, B, and 1, C, are typical of the rich mycelium that quickly develops in susceptible plants; and Fig. 1, D, is typical of the sparse mycelium within the coleoptile cells of the resistant plants.

SUMMARY AND CONCLUSION

In this paper, the writer reports and figures extensive penetration by the promycelium arising from the chlamydospore of *Ustilago levis*. No mycelial fusions were apparent in these cases. Does the dikaryophase arise from such a direct penetration? At present no evidence is available. There is also no present way of knowing that these promycelia produce sporulating infection. But it is true that these direct penetrations appear to be more numerous than might be expected.

The problem deserves further study. The technique of examining invasion hyphae needs refinement, and the progress of invasion hyphae should be studied by film, if possible.

In conclusion, it may be stated that under the environmental conditions reported here, penetration by the promycelium directly from the chlamydospore of *Ustilago levis,* race 7, without promycelial fusions, is more extensive than has been supposed. It is suggested that the story of the development of the oat smut chlamydospore on the coleoptile may be different in certain important respects from its development in culture.

Thanks are due to Dr. G. M. Reed for the materials with which this work has been carried on and for the advice he has given so generously.

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PATHOGENESIS OF APHANOMYCES COCHLIOIDES ON TAPROOTS OF THE SUGAR BEET[1]

W. F. Buchholtz and C. H. Meredith[2]

(Accepted for publication January 3, 1944)

Since the discovery that *Pythium debaryanum* Hesse is the principal cause of damping-off of very young sugar-beet seedlings in northern Iowa, the major role played by another pathogen in parasitizing older seedlings and plants with taproots of considerable size, in addition to young seedlings, under certain conditions in specific entire fields, has become increasingly evident.[3] Infection of plants at and beyond the seedling stage occurred in two adjacent fields on the Northern Iowa Experimental Association Farm (Fig. 1) in 1937 and in 1938. Isolation and inoculation trials indicated that *Aphanomyces cochlioides* Drechsler was the causal agent. Since some of the symptoms observed in the field and those induced by pure culture inoculations were on plants beyond the seedling stage, and, therefore, in addition to those hitherto attributed to *A. cochlioides,* they are herein briefly described.

SYMPTOMS

On young seedlings the symptoms were similar to those attributed to *Aphanomyces cochlioides* by Peters,[4] Edson,[5] and Drechsler.[6] Occasionally a general rapid necrosis of the entire root, hypocotyl, and the lower portion of the cotyledons of young seedlings occurred (Fig. 2, A). *A. cochlioides* apparently parasitizes very young seedlings less frequently than *Pythium debaryanum.* Furthermore, under field conditions, direct necrosis induced by *P. debaryanum* is restricted to that portion of seedlings below the soil surface, with brown rather than black discoloration of necrotic tissues.

On older seedlings or plants with 1 or 2 other leaves and with some secondary vascular enlargement there was necrosis of the cortex of roots and hypocotyls and of the cotyledonary petioles (Fig. 2, B). The necrotic tissues were black; in fact, this is the typical so-called "black-root" in northern Iowa. In this late seedling stage of development, the surfaces of hypocotyls and primary roots in *Aphanomyces cochlioides*-infested soil were rough and gray.

A later and very striking symptom induced by *Aphanomyces cochlioides* was a black disintegration of the taproot, which first became manifest in late

[1] Journal Paper No. J–1175 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 432.

[2] The writers are indebted to Dr. I. E. Melhus for aid and suggestions during the course of investigations and preparation of the manuscript.

[3] Buchholtz, W. F., and C. H. Meredith. A sugar-beet root rot caused by *Aphanomyces cochlioides.* (Abst.) Phytopath. 28: 4. 1938.

[4] Peters, L. Zur Kenntnis des Wurzelbrandes der Zuckerrübe. Ber. Deut. Bot. Gesell. 24: 323–329. 1906.

[5] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. Jour. Agr. Res. [U. S.] 4: 135–168. 1915.

[6] Drechsler, Charles. The beet water mold and several related root parasites. Jour. Agr. Res. [U. S.] 38: 309–361. 1929.

June and early July, when the thinned stands were recovering from the thinning operation (Fig. 2, C). The pathologic events leading to this condition are not entirely understood. There is likelihood of earlier infection and pathogenesis, for crowns of afflicted plants were considerably stunted and their lower leaves yellow in early stages of indirect necrosis. The central leaves were sometimes unusually stiff and glossy but dwarfed. Wilting in the afternoon of bright days, with subsequent recovery ("flagging") was common. The roots of such plants were undersized, black-tipped 3 to 6 inches below the soil line, with an excess of side roots developed apparently



FIG. 1. A field of sugar beets which was a complete failure because of "tip rot" caused by *Aphanomyces cochlioides*. Kanawha, Iowa, 1937. (Photograph by I. E. Melhus.)

in response to the destruction of the taproot (Fig. 2, C). A large majority of these side roots were black and shrivelled.

After mid-June, plants in infested soil either were killed or grew very slowly until late August, when there was some recovery and apparently satisfactory growth of many of the remaining plants. Even then, some plants underwent a progressive rot of the fleshy taproot at about the plow line. This rot was characterized by a first greenish-yellow, later light-brown and finally dark-brown, almost black, discoloration (Fig. 2, D). Infected tissues were slightly softened but still resisted the knife, and split rather than tore when cut. They appeared slightly water-soaked. When subject to desiccation such tissues finally shrivelled to a "tassel" of vascular elements (Fig. 2, E and F) as in the rot produced by *Phytophthora drechsleri*

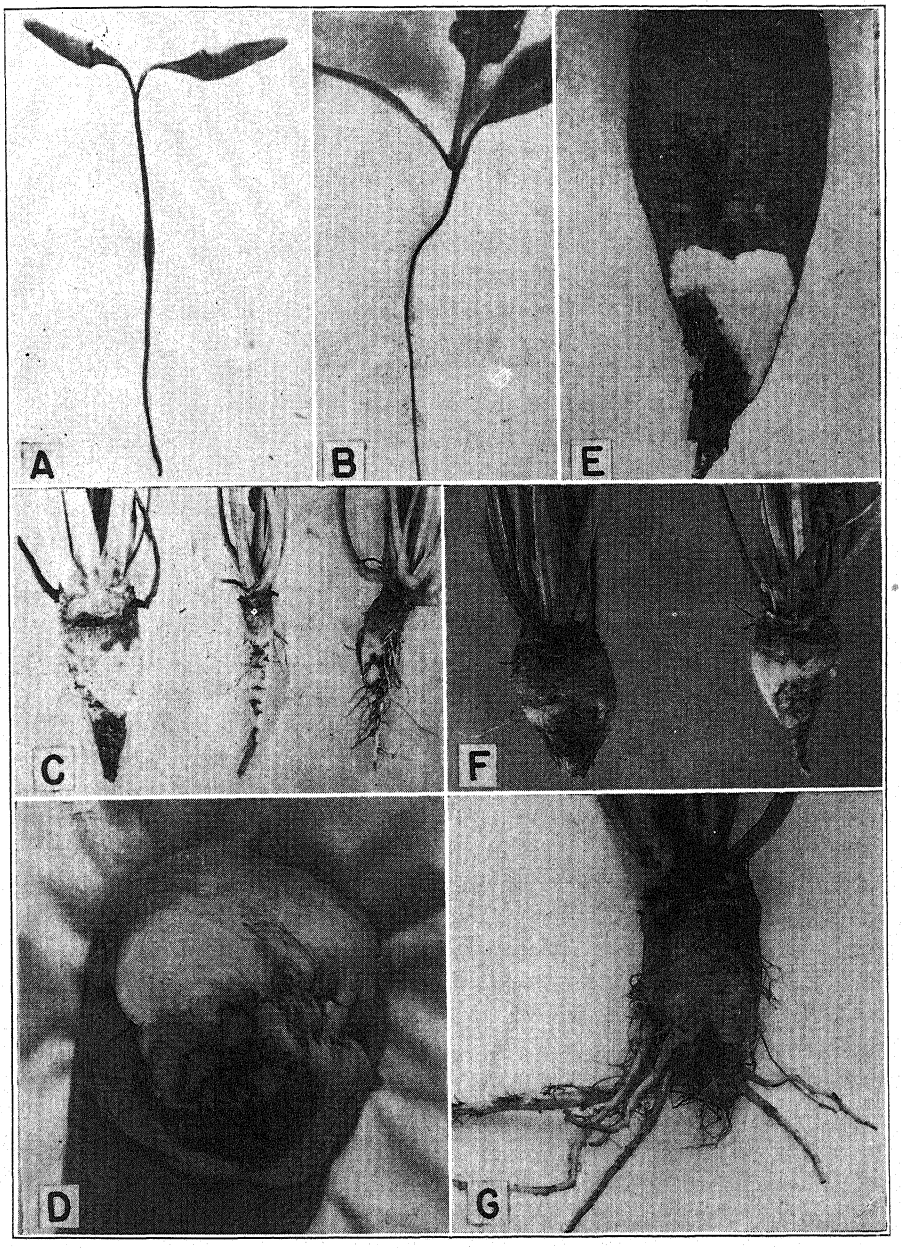


FIG. 2. A. Seedling that has undergone necrosis of the entire root, hypocotyl and lower portion of the cotyledons. B. Seedling with darkened hypocotyl and cotyledonary petioles ("black root"). This symptom is a reliable diagnostic characteristic of *Aphanomyces cochlioides* infection of beet seedlings. C. Tip-rotted beets about a month after thinning. The blackened taproots are typical as is the excessive development of side roots (also blackened) on the beet at the right. D. Typical watersoaked but firm, discolored area induced by *A. cochlioides* on large taproots. This root was exposed to an agar culture of *A. cochlioides*. E. Section cut through rotted, partially desiccated lower taproot. F. "Tassels" left on the taproot when tissues invaded by *A. cochlioides* become desiccated. G. An apparently "recovered" beet from a field that was a failure because of tip rot. Note the several horizontal roots rather than a taproot, and the blackened small roots.

Tucker.[7] Frequently the uppermost progress of rot of the fleshy taproot was in the two zones of lateral root protrusion. There was no distinct odor associated with rotted fleshy taproots.

Late in the season many remaining beets showed signs of previous infection and partial recovery. After the destruction of the original taproot, one or several of the side roots appeared to have achieved some degree of dominance and developed into a taproot, frequently in a more nearly horizontal than a vertical direction (Fig. 2, G). The vascular elements of the original taproot sometimes were in evidence.

Because the destruction of the taproot at about the plow line was the most common and characteristic symptom on plants beyond the seedling stage, "tip rot" became the common field designation for the disease.

Aphanomyces cochlioides appeared more or less regularly from plantings of diseased tissues on plain agar from the first time isolations were attempted in August, 1937. On August 21, two growths of *A. cochlioides* were detected in a series of plantings of dry, blackened side roots on plain agar. On August 23, *A. cochlioides* was detected in 6 of a series of isolations from rotten taproots. On August 31, 11 of 12 isolations from wet rotted taproots yielded *A. cochlioides,* and on September 21, 23 of 24 such isolations did also. Since then the same pathogen has been observed repeatedly and consistently in isolations from seedlings and plants of all sizes with the various types of symptoms described. Except for slightly smaller vegetative hyphae, the isolates obtained seemed to fall within the limits described by Drechsler.[8]

INOCULATION TRIALS

In 3 inoculation experiments in 1937, *Aphanomyces cochlioides* produced typical taproot-rot symptoms. In the first trial, September 11, corn-meal cultures of each of 2 isolates were introduced by means of plug wounds into 5 beets in the field. In the second, on September 20, each of 6 isolates were similarly introduced into 10 beets in the field. On October 14, when the beets in both experiments were lifted from the soil, all inoculated beets in the first experiment and 55 of 60 in the second were partially rotted. On September 29, each of 8 isolates was introduced by plug wounds into 3 lifted beets. The inoculated beets were stored in the greenhouse until November 1, when 23 had rotted. In all 3 experiments, the checks were not rotted, and *A. cochlioides* was recovered from the inoculated rotted beets. On two other occasions, sugar-beet roots were inoculated with isolates of *A. cochlioides* in the laboratory, and necrosis of tissues developed.

Four inoculation experiments were undertaken in 1938. Two were started in an April-planted field on July 12 and August 5. Ten plugged and 5 unwounded beets were exposed to each of 6 isolates of *Aphanomyces cochlioides.* Two similar unexposed series served as checks. The beets were lifted and examined on August 19. From the July 12 inoculations, only 2

[7] Tompkins, C. M., B. L. Richards, C. M. Tucker, and M. W. Gardner. Phytophthora rot of sugar beet. Jour. Agr. Res. [U. S.] 52: 205–216. 1936.

[8] See footnote 6.

of 60 plugged beets showed typical rotting; none of the uninjured beets showed any evidence of infection. From the August 5 inoculations, 2 isolates typically rotted all 10 plugged beets, a third isolate rotted 5 of 10 plugged beets, a fourth isolate rotted 1 of 10 plugged beets, the other 2 isolates rotted none. One of the 30 unwounded, exposed beets showed evidence of typical rotting by *A. cochlioides*. One of the beets that rotted as a result of exposure to a virulent isolate is shown in figure 2, D.

In a third similar experiment, started August 19, only the 4 apparently most virulent isolates were used. One isolate, which rotted an unwounded beet in the August 5 inoculation, typically rotted all 10 plugged beets; the 3 other isolates rotted 8, 7, and 4 plugged beets, respectively. No unwounded exposed beets were rotted. All checks remained healthy.

A fourth experiment was begun on August 18. Three sets of 4 injured (3 scratches with knife blade) and 4 uninjured beets of 4 ages (planted March 29, April 12, May 15, June 4) were exposed to 2 virulent isolates of *Aphanomyces cochlioides*. On September 9 the beets were pulled and the following observations made: Five of 16 injured, exposed beets from the two first plantings were slightly rotted on the surface only. Four of 8 injured beets in the third planting were similarly surface-rotted; one other had typical "tip rot." In the first 3 plantings, no uninjured exposed beets showed any evidence of infection as indicated by rotting. Of the youngest beets (June 4 planting) 7 injured and 7 uninjured roots showed some rot. Obviously roots of the last planted beets were rotted more after exposure to *A. cochlioides* than those of the larger beets in the first 3 plantings. All roots exposed to sterile agar were healthy.

In several trials, isolates of *Aphanomyces cochlioides,* when added to steamed soil, caused damping-off of beet seedlings growing in such soil. The invaded necrotic seedling tissues invariably were black, and the cotyledonary petioles were typically invaded (Fig. 2, A). In each field inoculation trial in 1938, and in two soil infestation trials in the greenhouse, *A. cochlioides* was recovered from one or more rotted roots or damped-off seedlings.

SUMMARY

The pathogenic effects of *Aphanomyces cochlioides* on sugar beet plants at and beyond the young seedling stage were observed in northern Iowa and are recorded. The symptoms were necrosis and blackening of hypocotyls, cotyledonary petioles and roots of seedlings; blackening and disintegration of the taproot 3 to 6 inches below the soil line at thinning time, with wilting and indirect necrosis of leaves; necrosis and blackening of excessive side roots; a greenish-yellow to brown or black semi-soft rot of the lower end of large taproots. *Aphanomyces cochlioides* was isolated frequently from such tissues.

Inoculation experiments with pure cultures induced symptoms similar to those observed in the field. In one inoculation trial with beets of 4 ages, young roots seemed to be more susceptible than older roots.

THE SEQUENCE OF INFECTION OF A SEEDLING STAND OF SUGAR BEETS BY PYTHIUM DEBARYANUM AND APHANOMYCES COCHLIOIDES[1, 2]

W. F. BUCHHOLTZ

(Accepted for publication January 13, 1944)

The role of *Pythium debaryanum* Hesse in destroying germinating seed and young seedlings of sugar beets in northern Iowa is well known.[3] Seed treatment also is recognized as valuable and is practiced universally among sugar beet growers. Although seed treatment has been effective in establishing good stand of young seedlings, these stands often have failed soon after their establishment. It is known that *Aphanomyces cochlioides* Drechsler can attack the roots of older seedlings and of larger plants.[4]

In this presentation an attempt is made to delineate the roles of *Pythium debaryanum* and *Aphanomyces cochlioides* in destroying seedling stands in northern Iowa soils and to evaluate seed treatment as a control for each.

METHODS

In September, 1938, samples of Clarion loam and Webster loam soils were taken from a field that had been in alfalfa for 3 years and that had not grown beets since 1932. Beets growing in this field in 1939 showed no evidence of infection by *Aphanomyces cochlioides,* although the seedling stand loss from *Pythium debaryanum* was heavy on the Clarion loam and light on the Webster loam. A comparable sample of Webster loam was taken from an adjacent field in which beets were heavily diseased at all ages by *A. cochlioides* and considerable numbers of young seedlings destroyed by *P. debaryanum.* The pH values of the 3 samples were 5.5, 6.6, and 6.3, respectively. The samples were stored in the greenhouse as taken from the field in galvanized tin cans, covered but not sealed. Normal greenhouse temperatures prevailed, and the samples did not become dry.

Five 4-inch pots were filled from each of the 3 different soil samples, in January, 1939. A row of 10 nontreated and a row of 10 treated (New Improved Ceresan at the rate of 5 oz. per 100 lb. of seed) clusters of American No. 1 seed were planted in each pot. In addition to the above samples, 5 pots of the Webster loam infested with *Pythium debaryanum* and *Aphanomyces cochlioides* were steamed and likewise planted. Germination and number of diseased seedlings in each pot were recorded daily for 28 days. Diseased seedlings were removed every day, washed, and laid on

[1] Journal Paper No. J–1171 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 432.

[2] The writer is indebted to Dr. I. E. Melhus for helpful suggestions in connection with the investigation and in the preparation of the manuscript.

[3] Buchholtz, W. F. Factors influencing the pathogenicity of *Pythium debaryanum* on sugar beet seedlings. Phytopath. 28: 448–475. 1938.

[4] Buchholtz, W. F., and C. H. Meredith. Pathogenesis of *Aphanomyces cochlioides* on taproots of sugar beet. Phytopath. 34: 485–489. 1944.

plain agar. The pathogens grew out in 24 to 48 hours and were identified and recorded. Of 245 seedlings becoming diseased, diagnosis was successful with all but 4. *P. debaryanum* produced sphaerosporangia and occasional oogonia, antheridia, and oospores in 48 hours. *A. cochlioides* exhibited typical coarse, tortuous mycelium and occasionally the first stage of zoospore formation in 8 to 24 hours.

EXPERIMENTAL RESULTS

In steamed soil the rate and extent of germination of treated and nontreated seed were about the same. Germination was nearly complete 11

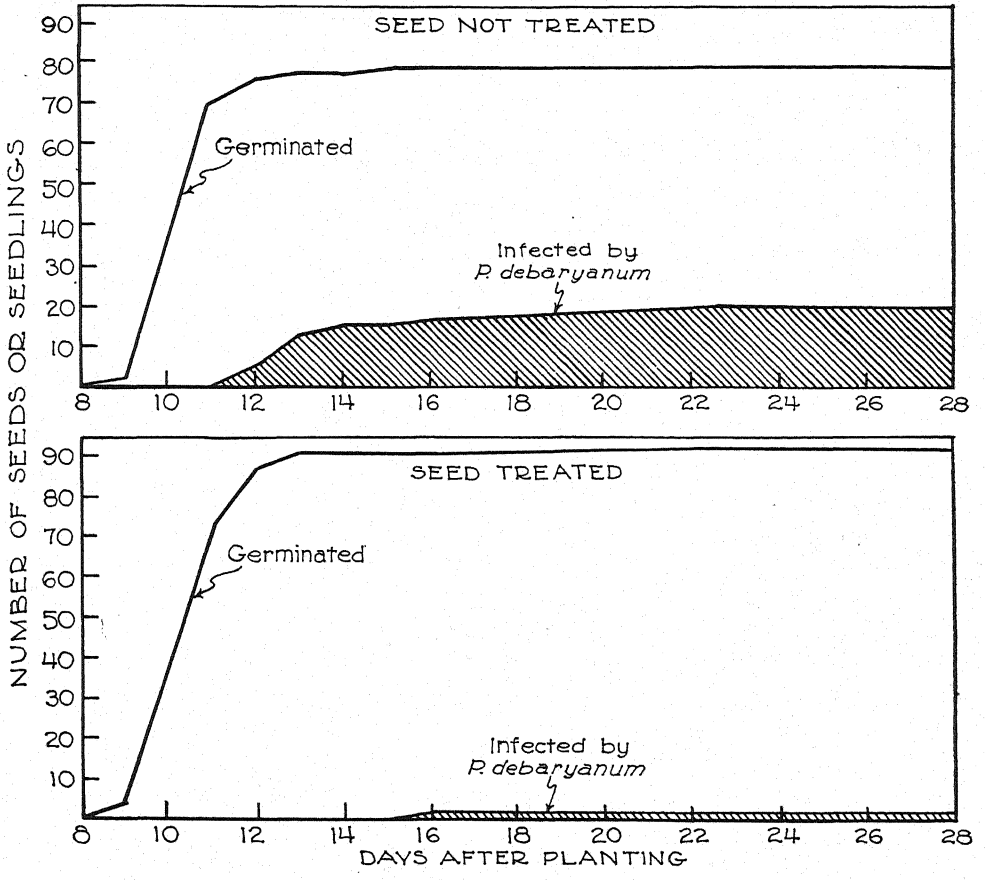


FIG. 1. The number of seeds germinated and seedlings infected daily from 50 nontreated and 50 treated seed clusters planted in the greenhouse in Webster loam lightly infested with *Pythium debaryanum*.

days after planting, and the final numbers of seedlings from 50 nontreated and 50 treated seed clusters were 90 and 86, respectively. No seedlings were diseased (Fig. 4, A). This lot of seed apparently was free from *Phoma betae*.

In soil lightly infested with *Pythium debaryanum* (Webster loam, alfalfa sod), germination was nearly completed 12 days after planting (Fig. 1).

Germination was less from nontreated than from treated seed, which probably was due to interruption of the germination of nontreated seed by *P. debaryanum*. Loss of seedlings from infection by *P. debaryanum* occurred soon after germination, and there was little or no loss beyond the 14th day after planting, when there were 63 and 91 healthy seedlings remaining from nontreated and treated seed, respectively (Fig. 4, B). Twenty seedlings grown from the nontreated seed were killed. Since ap-

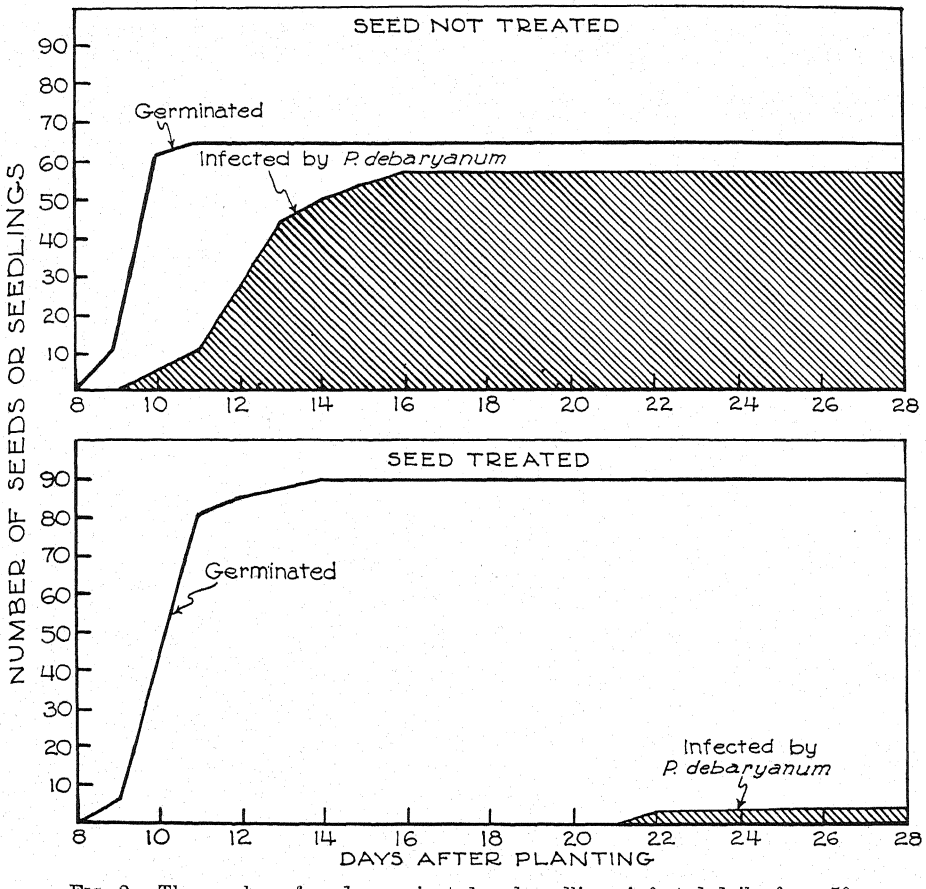


FIG. 2. The number of seeds germinated and seedlings infected daily from 50 nontreated and 50 treated seed clusters planted in the greenhouse in Clarion loam heavily infested with *Pythium debaryanum*.

proximately 10 germinating seeds probably were destroyed, the loss was $\frac{1}{3}$ the expected stand of approximately 90. Only 1 seedling from treated seed was destroyed, also by *P. debaryanum*.

In soil heavily infested with *Pythium debaryanum* (Clarion loam, alfalfa sod), germination was nearly completed 11 days after planting (Fig. 2). A total of 65 seedlings resulted from nontreated seed and 90 from treated seed, a difference of 25 as compared with a difference of 13 in the Webster loam, lightly infested with *P. debaryanum*. Here again the loss of seed-

lings grown from nontreated seed occurred soon after germination. Most of the loss had taken place by the 14th day and none beyond the 16th day after planting, when there were 7 and 90 healthy seedlings remaining from nontreated and treated seed, respectively (Fig. 4, C). Fifty-seven seedlings from nontreated seed were infected by *P. debaryanum*. Since 25 germinating seeds probably were destroyed, there was a total loss of 82 seeds and seedlings, or fully 90 per cent of the probable number of live seeds. Only 4 seedlings from treated seed were infected by *P. debaryanum*.

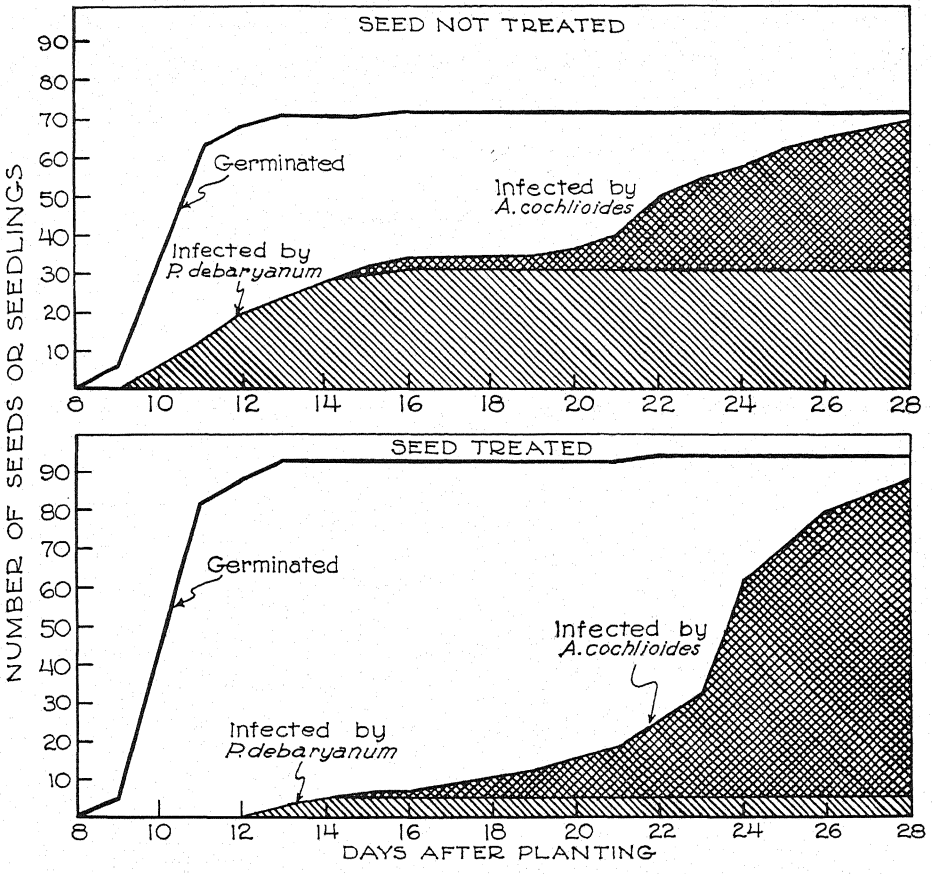


FIG. 3. The number of seeds germinated and seedlings infected daily from 50 treated and 50 nontreated seed clusters planted in the greenhouse in Webster loam heavily infested with *Pythium debaryanum* and *Aphanomyces cochlioides*.

In soil infested with *Pythium debaryanum* and *Aphanomyces cochlioides* (Webster loam), germination was nearly completed 12 days after planting (Fig. 3). There were, in all, 72 seedlings from nontreated seed and 94 from treated seed, a difference of 22 as compared with 13 in the lightly infested Webster loam and 25 in the heavily infested Clarion loam. Loss of seedlings from nontreated seed by *P. debaryanum* infection again was rapid. Nearly all infection had occurred by the 15th day after planting, and none occurred

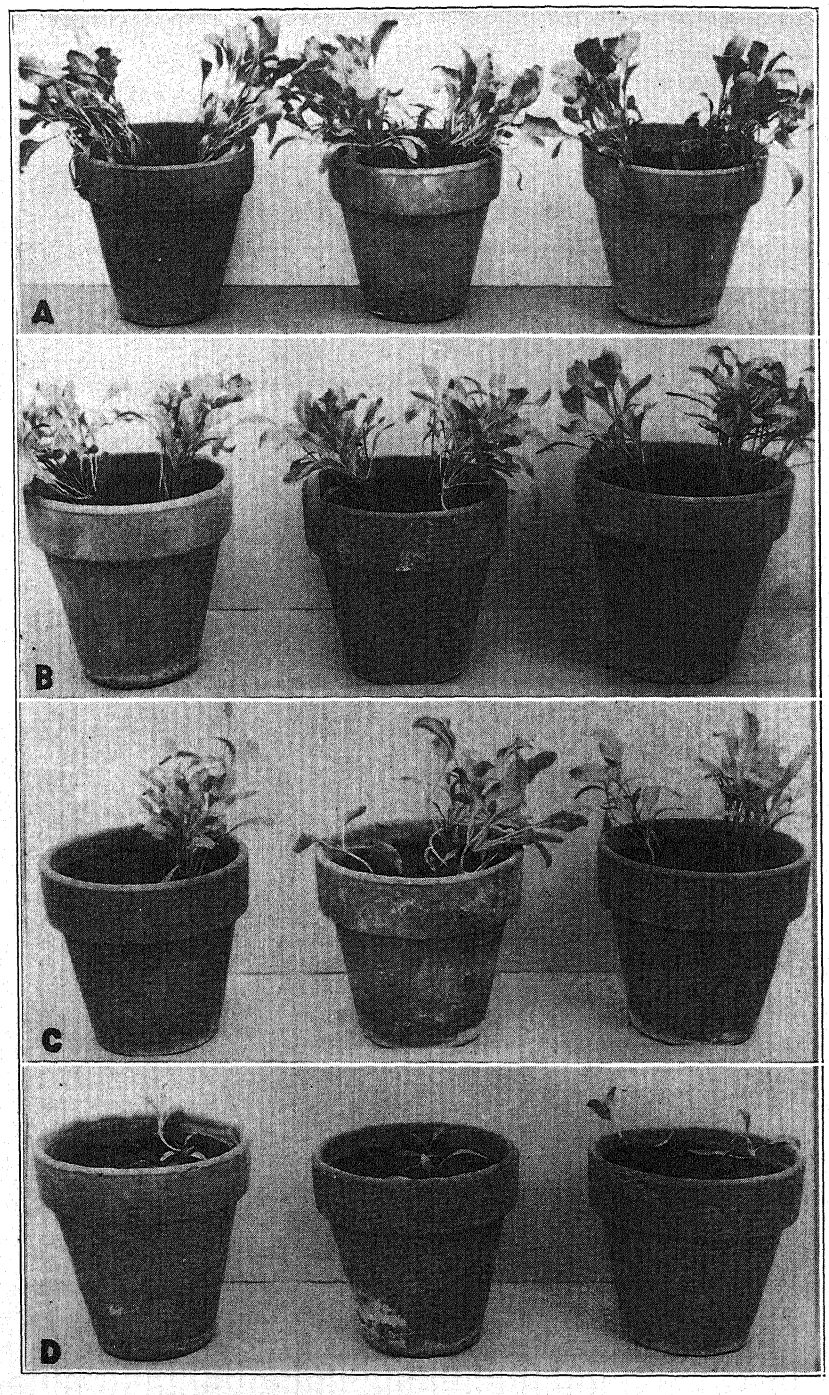


FIG. 4. Typical final stands of sugar beet seedlings grown in (A) steamed Webster loam, (B) Webster loam lightly infested with *Pythium debaryanum*, (C) Clarion loam heavily infested with *P. debaryanum*, and (D) Webster loam infested by *P. debaryanum* and *Aphanomyces cochlioides*. In each pot the left row is from nontreated seed, the right row from treated seed.

after the 16th day. Thirty-one seedlings from nontreated seed were infected by *P. debaryanum* in addition to the 22 germinating seeds probably destroyed by this pathogen. Thus, a total of 53 seeds and seedlings, or nearly 60 per cent, probably were killed by *P. debaryanum.* The interruption in germination in this case was assumed to have been caused by *P. debaryanum,* since the loss of seedlings from infection by *P. debaryanum* had ceased by the time the first seedlings were visibly infected with *Aphanomyces cochlioides.* Only 5 seedlings from treated seed were infected by *P. debaryanum,* all by the 15th day after planting.

In stands from treated and nontreated seed loss of seedlings from infection by *Aphanomyces cochlioides* began on the 15th day after planting and continued until the 28th day after planting, when there remained only 1 healthy seedling from nontreated seed and 3 from treated seed (Fig. 4, D).

FIELD OBSERVATIONS

In the spring of 1939, plantings of treated and nontreated seed were made in the field on the areas from which the soil samples had been taken for the greenhouse tests. Isolation from every infected seedling, as in the greenhouse test, was impossible because many died and dried up before they were picked up, even though the stands were observed daily. Isolations were made daily, however, from typically diseased seedlings from treated and nontreated seed in the 3 areas. In general, as in the greenhouse tests, stands from nontreated seed in the heavily infested Clarion loam and in the lightly infested Webster loam were reduced more by *Pythium debaryanum* than were the stands from treated seed in the same areas. In the soil infested with *Aphanomyces cochlioides,* however, nearly the entire stand, whether from treated or nontreated seed, became infected by this pathogen.

The general infection of beet stands in commercial fields infested with *Aphanomyces cochlioides* is a common occurrence. In many fields stands of young seedlings may be excellent, which, in some cases, doubtless is the result of protection from *Pythium debaryanum* by seed treatment. It has been repeatedly observed, however, that in field stands, as in the greenhouse cultures, *A. cochlioides* destroys plants that have developed beyond the very young seedling stage, at a time when a seed disinfectant and protectant cannot be expected to persist and protect the growing plant.

SUMMARY

Pythium debaryanum killed 33 per cent of the sugar beet seedlings grown from nontreated seed in lightly infested soil, 90 per cent in heavily infested soil, and 60 per cent in soil in which *P. debaryanum* and *Aphanomyces cochlioides* occurred together. The killing of seedlings by *P. debaryanum* was nearly completed 15 days after planting. Very few of the seedlings that developed from treated seed were killed by *P. debaryanum.*

Aphanomyces cochlioides infection began when the seedlings ceased

dying from *Pythium debaryanum* infection, about 15 days after planting. By the 28th day after planting nearly all the remaining live seedlings, whether from treated or nontreated seed, had been attacked by *A. cochlioides*.

Pythium debaryanum took its toll quickly and was readily controlled by seed treatment, whereas *Aphanomyces cochlioides* attacked older seedlings and was not controlled by seed treatment.

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ORNITHOGALUM MOSAIC

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(Accepted for publication January 12, 1944)

A double-flowered variety of *Ornithogalum thyrsoides* Jacq. (Liliaceae) was received from a grower in Oregon in 1940. All plants from the original sample of bulbils exhibited mild green mottling in leaves and prominent light- and dark-green blotching in flower stalks. Seedlings of the same species grown from commercial seed were single-flowered and free from mottling. The disease in the Oregon specimens was investigated to determine its relation to the virus diseases of lily, tulip, hyacinth, and onion.

The only previous record of mosaic in *Ornithogalum* known to the writers is that by Nance[3] from Oklahoma in plants under the name of *Ornithogalum aureum*. The writers' material included diseased *Hyacinthus orientalis* L., variety Yellow Hammer, *Galtonia candicans* Decne., and *O. thyrsoides* from Oregon; and *Lachenalia* sp. and *O. thyrsoides* var. *aureum* Ait. from Alabama. Conspicuous mottling was noted also in leaves of red squill (*Urginea maritima* (L.) Baker) grown at the Plant Introduction Station, Glenn Dale, Maryland, from bulbs of Mediterranean origin. Mosaic is apparently widespread in a number of genera of the squill tribe (Scilleae) of the Liliaceae, but affected plants are evidently salable.

SYMPTOMS

In *Ornithogalum thyrsoides* young leaves show a fine mottling of light- and dark-green, which becomes gray or yellow and more conspicuous as the leaves mature (Fig. 1, A). Flower stalks are sometimes boldly marked with sharply contrasting light- and dark-green blotches (Fig. 1, B). In the white perianth segments, longitudinal thin streaks often appear, resembling the streaks in narcissus flowers accompanying narcissus mosaic. The mosaic patterns noted in the leaves of *Ornithogalum* and *Lachenalia* in Alabama, and in *Galtonia* and *Hyacinthus* in Oregon, were similar. In *Galtonia* grown in the greenhouse the symptoms were much milder than those noted in the open in Oregon.

TRANSMISSIBILITY

Methods

Plants for inoculation were grown from seed in a screened greenhouse kept free from sucking insects by frequent fumigations with nicotine. No natural infections in control plants were detected during the experiments of three seasons. Mechanical transfers were made by rubbing extracted sap

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[3] Nance, N. W. *Ornithogalum aureum*. *In* Diseases of plants in the United States in 1939. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Sup. 128, p. 368. 1940. [Processed.]

on young leaves previously dusted with carborundum powder. Aphids were reared in a separate insectary greenhouse. A nonviruliferous colony of *Aphis gossypii* Glover was maintained on chrysanthemum (*Chrysanthemum hortorum* L. H. Bailey), colonies of *Myzus persicae* Sulz. and of *Macrosiphum solanifolii* Ashm. on snapdragon (*Antirrhinum majus* L.), and colonies of *Macrosiphum lilii* Monell and of *Myzus circumflexus* Buckt. on virus-free seedlings of Easter lily (*Lilium longiflorum* Thunb.).

In early experiments aphids were transferred by brush to separate infected source plants in cages, where they were left for 24 hours or, if on

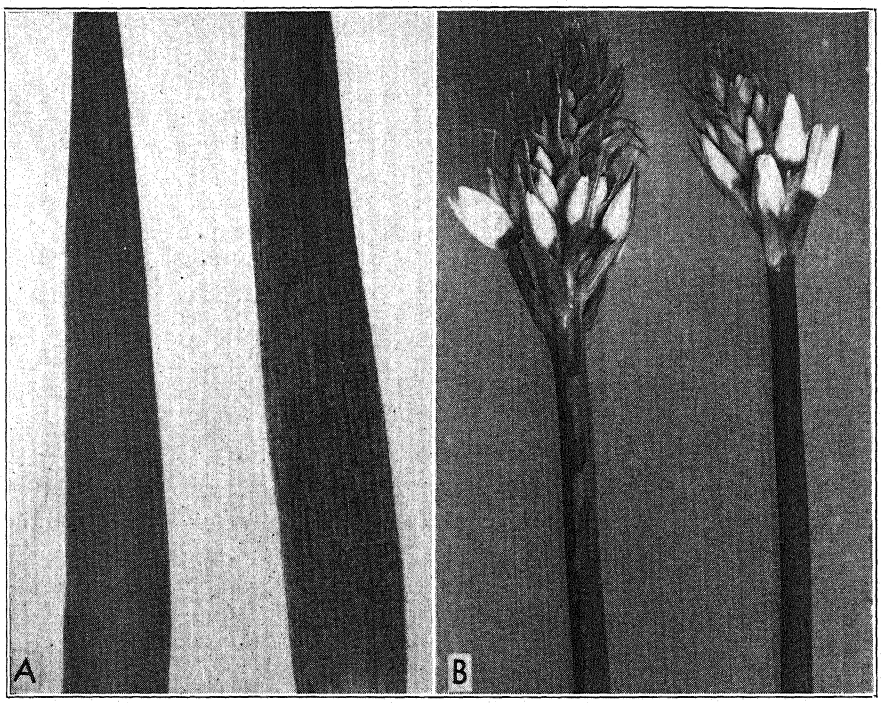


FIG. 1. Symptoms caused by Ornithogalum-mosaic virus in *Ornithogalum thyrsoides*. A. Leaf mottling, transmitted by *Myzus persicae*, in comparison with leaf of a control plant. B. Blotching of a flower stalk, transmitted by *Macrosiphum solanifolii*, in comparison with control.

favorable food plants, until needed. In later experiments the aphids were transferred to pieces of leaves taken from the source plants and placed in Petri dishes, where they fed for 1 to 3 hours. In transmission trials pieces of leaves of the source plant on which aphids were feeding were transferred to pieces of paper resting on the foliage of plants that had been brought to the insectary greenhouse and caged for this purpose. After 7 to 32 days on such test plants the aphids were destroyed by spraying or dipping in a pyrethrum preparation. The test plants were then returned to the insect-free greenhouse for observation.

Aphis gossypii fed readily and increased freely on *Ornithogalum* and

Easter lily. *Myzus persicae* from snapdragon and *M. circumflexus* from lily were readily transferred to *Ornithogalum*, where they fed on leaves and on flower parts in various stages of development. *Macrosiphum solanifolii* fed readily and established colonies on hyacinth and *Ornithogalum;* fed for several days, but failed to establish colonies on *Galtonia candicans, Agapanthus africanus* (L.) Hoffmg., and *Pancratium maritimum* L. *Macrosiphum lilii* bred continuously in large numbers on *Ornithogalum* and on Easter lily, but fed sparingly on *Zephyranthes* sp. All aphids in these tests died within 2 days on onion (*Allium cepa* L.).

Experimental Results

Experimental transfers of the virus from *Ornithogalum thyrsoides* to seedlings of this species are listed in table 1. Transfer by sap, using the carborundum-leaf-rubbing technique, proved difficult. Three of 8 trials

TABLE 1.—*Transmission of Ornithogalum mosaic virus from Ornithogalum thyrsoides to O. thyrsoides seedlings*

Method of transfer	Number of aphids per trial	Number of trials	Proportion of plants infected[a]	Minimum incubation period[b]
				Days
Mechanical	.....	8	6/75	32
Aphis gossypii	105	1	5/5	17
Macrosiphum lilii	265	1	5/5	10
Macrosiphum solanifolii	75	1	5/5	31
Myzus circumflexus	40	2	4/10	34
Myzus persicae	20–300	10	46/46	10

[a] Number of plants infected over number exposed.
[b] Interval between inoculation and appearance of symptoms.

were failures, and in the best trial only 2 of 10 plants became infected. No advantage was found in extracting the sap with phosphate buffers adjusted to pH 6, 7, or 8, or with 1 per cent dextrose or 0.85 per cent NaCl. No attempt was made to determine properties of the virus because of this low degree of success in transfer by sap.

All 5 species of aphids tested transferred the virus, and all except *Myzus circumflexus* with high efficiency. Uniform symptoms resulted from transfer by the several species of aphids and by sap, indicating that a single virus is concerned. No great importance is attached to differences in the incubation period, as daily records were not made on this point, and growth rates following inoculation varied. Under favorable conditions the symptoms may appear within 10 days following aphid transfer; much longer intervals seem to be characteristic of mechanical transfer.

The virus has been transmitted also to *Ornithogalum thyrsoides* by *Myzus persicae* from a yellow-flowered variety, *O. thyrsoides* var. *aureum*, and also from *Lachenalia*, variety Rector of Cawston, from Alabama. Symptoms typical of Ornithogalum mosaic were recognized after 18 days in each of

these 2 tests, all exposed plants becoming infected. *Myzus persicae* transferred the virus from naturally infected *Galtonia candicans* from Oregon in each of 2 trials. From mottled hyacinth variety Yellow Hammer, from Oregon, *Myzus persicae* infected 6 of 10 *Ornithogalum* plants in 1 trial. In the first subtransfer from this set to other *Ornithogalum* seedlings, 4 of 10 plants were infected; but 2 later serial transfers induced mottle in all plants exposed. This vector failed to transmit the disease from Yellow Hammer hyacinth in a second trial, and failed to transfer virus from hyacinth varieties Grand Maître and L'Innocence that showed mild mottling in leaves and flower stalks. *Myzus persicae* failed to transmit a virus from mottled red squill (*Urginea*) to *Ornithogalum* in 1 trial. *Macrosiphum solanifolii* transferred the virus from Yellow Hammer hyacinth to 1 of 5 plants of *Ornithogalum* in 1 trial. The symptoms induced in *O. thyrsoides* by the virus from *Lachenalia, Galtonia,* and *Hyacinthus* appear identical with those of the Ornithogalum mosaic virus occurring in nature.

Attempts to transfer Ornithogalum-mosaic virus to healthy seedlings of other members of the Scilleae have for the most part failed or proved inconclusive. Viruliferous *Myzus persicae* colonized on seedlings of *Eucomis undulata* Ait. produced after 6 days conspicuous angular yellow blotches in 2 of 3 plants. These supposed local lesions persisted for months, but systemic symptoms did not appear, and the virus was not returned to *Ornithogalum thyrsoides* by *M. persicae* in a trial 1 month after the original test. No symptoms whatever resulted from a second transfer to *Eucomis* under similar conditions.

No definite symptoms resulted from attempted transfers of the virus to Galtonia by *Myzus persicae* and by *Macrosiphum solanifolii,* and a subtransfer back to *O. thyrsoides* by *Myzus persicae* 2 months after exposure was also without effect. *Myzus persicae* failed to induce symptoms in seedlings of *Camassia leichtlinii* (Baker) S. Wats., *Hyacinthus azureus* (Fenzl.) Baker, *Muscari botryoides* Mill. variety Heavenly Blue, and *Scilla peruviana* L., and failed to return the virus from inoculated plants of *Camassia, Muscari,* and *Scilla.*

In other trials efficient vector species, chiefly *Myzus persicae,* failed to transmit Ornithogalum-mosaic virus to the following plants: Amaryllidaceae—*Pancratium maritimum, Zephyranthes* sp.; Iridaceae—*Tritonia crocata* (L.) Ker.; Liliaceae—*Agapanthus africanus, Allium cepa* (onion), varieties California Early Red, Utah Sweet Spanish, *A. cernuum* Roth., *A. fistulosum* L., *A. porrum* L., *Gloriosa rothschildiana* O'Brien, *Lilium formosanum* Stapf., and *L. longiflorum.* Mechanical inoculations of *L. formosanum, L. longiflorum, Tulipa gesneriana* L., variety Clara Butt, and *Nicotiana tabacum* L. variety Samsun (Turkish), resulted in no infection.

Attempts to transfer the virus from *Ornithogalum* to tulip by aphids have yielded inconsistent results. Flower breaks of types produced by tulip viruses resulted from 3 transfers of *Myzus persicae,* but not from 4 other trials. The virus was not returned from tulip to *Ornithogalum* by *M.*

persicae in either of 2 trials conducted. *Myzus circumflexus,* a proved vector of Ornithogalum mosaic virus, failed to carry this virus from *Ornithogalum* to tulip in one experiment. Inasmuch as available stocks of tulips carry some breaks, and as *M. persicae* is an efficient vector of tulip-break viruses it is possible that this species has accomplished the spread of tulip-break viruses occasionally present as contaminants, rather than the apparent transfer of Ornithogalum mosaic virus to tulip. The susceptibility of tulip to this virus must, therefore, be considered in doubt.

The following inoculations from diseased plants to *Ornithogalum thyrsoides* seedlings were without effect: Cucumber mosaic by sap (subinoculations to tobacco negative); iris mosaic by *Macrosiphum solanifolii;* mottle of Easter lily by *Myzus persicae* and also by sap (subinoculation to *Lilium formosanum* negative); Easter lily necrotic fleck complex by *Aphis gossypii* (subinoculations to tobacco and *L. formosanum* negative); narcissus white-streak[4] by *M. persicae;* and onion yellow dwarf[5] by *M. persicae.* No effect resulted from transfer of tulip virus 1, tulip virus 2, and lily latent virus from *L. formosanum* to *Ornithogalum* by *M. persicae.* The methods of transfer used have been demonstrated to be effective for the viruses concerned with the exception of that of narcissus white streak, which is not known to be carried by *M. persicae.*

Also transmissible to *Ornithogalum thyrsoides,* in addition to the virus described herein, is a recently encountered virulent form of the mottle virus of Easter lily, distinguishable from the usual mottle by the reaction of *Ornithogalum* and also by severe deforming symptoms in Easter lily. The virulent mottle virus is also transmissible by *Myzus persicae,* but induces symptoms recognizably different from those of Ornithogalum mosaic in *O. thyrsoides* as discussed in a separate publication.[6]

The virus from Yellow Hammer hyacinth, shown above to be transmissible to *Ornithogalum* by aphids, and considered identical with Ornithogalum-mosaic virus in the writers' experience, was not transmitted to Clara Butt tulips, to Easter lily seedlings, or to Croft Easter lilies by *Myzus persicae.* Mechanical inoculations from Yellow Hammer hyacinth to Clara Butt tulip, to Easter lily seedlings, to *Lilium formosanum,* and to Turkish tobacco were similarly negative. McWhorter[7] writes that the Yellow Hammer hyacinth, purchased from a commercial grower, showed characteristic mottling when first grown, and for 10 years thereafter. He was unable by mechanical methods to transfer a virus from this stock to tulip, to *Trifolium incarnatum* L., to *Vicia faba* L., or to an apparently virus-free blue-flowered variety of hyacinth. He concluded that a transitory mottling induced in some Easter lily seedlings was a shock reaction. He failed to produce any symptoms in hyacinths on inoculation with Tulip virus 1 from tulip, or with lily-mottle viruses of the tulip group from *Lilium candidum* L. and *L.*

[4] Source material from F A. Haasis.
[5] Source material from F. P. McWhorter.
[6] Brierley, Philip, and Floyd F. Smith. Studies on lily virus diseases: the mottle group. (In press.)
[7] Personal communication from F. P. McWhorter.

martagon L. He, therefore, agrees with the writers that the virus from Yellow Hammer hyacinth is not transmissible to lily or tulip, and that the usual lily and tulip viruses do not affect hyacinths.

DISCUSSION

Atanasoff[8] described mosaic symptoms in species of *Hyacinthus* and of *Muscari* that he considered due to "the mosaic disease of bulb plants," affecting various other members of the Liliaceae, Amaryllidaceae, and Iridaceae. He reported further that "the mosaic disease of tulips, hyacinths, and narcissi passes easily to narcissus and tulip." This implication that various distantly related bulbous plants are subject to a common mosaic disease has not been supported by more recent studies of Haasis[9] and of the writers.

All proved suscepts of the Ornithogalum-mosaic virus are members of the tribe Scilleae of the Liliaceae. Even within this tribe several species appear to be refractory to infection, including the Heavenly Blue variety of *Muscari botryoides*. Three species of *Muscari* were reported subject to mosaic by Atanasoff.[8] Perhaps the present failure to infect other genera of the Scilleae with this virus should be discounted in view of the difficulty of producing infection in *Galtonia candicans*, which seems subject to the same virus in nature. No explanation is evident for this difficulty of intergeneric transfer by vectors highly efficient in transfer within certain species. It was observed, however, that aphids transferred from *Ornithogalum*, a favorable host plant, to *Galtonia* failed to settle down and feed, at least for some time. It seems possible that during this interval the insects might lose the virus and fail to cause infection even though they feed later. Transfers from hyacinth Yellow Hammer to *Ornithogalum* were also erratic. A virus apparently identical with Ornithogalum-mosaic virus occurs in hyacinths, but it is uncertain whether this virus is identical with that of the hyacinth mosaic of other writers.[8]

Ornithogalum-mosaic virus shows no indication of relationship with the viruses of *Allium* or with those of the Iridaceae. Some relationship with the tulip-breaking group that causes mosaic diseases of tulip and lily is suggested. Symptoms occasionally induced in tulip on transfer from *Ornithogalum* by *Myzus persicae*, while not accepted as wholly conclusive, resemble the effects of some of the tulip-breaking group. One of the tulip-breaking group (virulent mottle of Easter lily) has been shown to affect *Ornithogalum*. On the other hand *Macrosiphum lilii* and *Myzus circumflexus*, proved vectors of Ornithogalum-mosaic virus, have not transferred viruses of the tulip-breaking group in the writers' tests. If a close relationship exists between the Ornithogalum-mosaic virus and the tulip-breaking viruses consistent transfer between tulip and Ornithogalum might be ex-

[8] Atanasoff, D. Mosaic disease of flower bulb plants. Bull. Soc. Bot. de Bulgarie 2: 51–60. 1928.

[9] Haasis, F. A. Studies on narcissus mosaic. New York (Cornell) Agr. Exp. Stat. Mem. 224. 22 pp. 1939.

pected by *M. persicae,* which is an efficient vector of both, and feeds freely on both *Ornithogalum* and tulip.

The identity of Ornithogalum-mosaic virus with the long-known but inadequately described hyacinth mosaic virus cannot be established without return inoculation to virus-free hyacinths. Virus-free hyacinths presumably can be produced from seed, but this procedure is too time-consuming to be undertaken at present. Since Ornithogalum-mosaic virus is considered sufficiently distinct from the previously known viruses to stand as a separate entity, the technical name *Marmor scillearum* is proposed, to indicate that it is known to occur only in certain members of the tribe Scilleae of the family Liliaceae.

Control of mosaic in *Galtonia, Hyacinthus, Lachenalia,* and *Ornithogalum* promises to be difficult. In view of the number of effective vector species, culture of seedlings at a distance from bulb-propagated suscepts offers most promise of success. This suggestion is supported by the fact that no evidence of seed transmission of Ornithogalum-mosaic virus was detected in 143 seedlings of *Ornithogalum thyrsoides* from mosaic-affected parents. It is of practical value to know that this common disease of minor bulbs is no menace to lilies or to onions. *Ornithogalum thyrsoides,* easily grown from seed, is of some technical interest as a test plant for viruses occurring in other monocotyledonous plants.

SUMMARY

A mosaic disease of *Ornithogalum* is described. The virus is transmissible with difficulty by sap, with a high degree of efficiency by *Aphis gossypii, Macrosiphum lilii, Macrosiphum solanifolii,* and *Myzus persicae,* and with somewhat lower efficiency by *Myzus circumflexus.* Viruses indistinguishable from this, at least in *Ornithogalum,* have been transferred from naturally infected plants of *Galtonia candicans,* hyacinth, and *Lachenalia* sp. The proved host range of the virus includes only species of these genera, and possibly of *Eucomis,* all members of the tribe Scilleae of the Liliaceae. The common name Ornithogalum-mosaic virus and the technical name **Marmor scillearum** are proposed.

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PHYTOPATHOLOGICAL NOTES

Reaction of Lycopersicon spp. to Spotted Wilt.—Spotted wilt of tomatoes is sufficiently widespread and destructive in certain parts of the world to justify a search for resistance. Investigations by D. R. Porter (unpublished), made prior to the experiments reported herein, have established that one strain of *Lycopersicon pimpinellifolium* is resistant to spotted wilt under conditions of natural infection. Wenholz[1] has reported briefly the resistance of this species and of an unnamed Peruvian type. The following are additional observations on the resistance to spotted wilt of certain tomato species. This report is based on field infection only and plants were classified as to disease reaction solely on the basis of visible symptoms.

During the summers of 1942 and 1943, plots were located at the trial grounds of a seed company at Salinas, California, where extensive plantings of ornamentals provide an excellent source of spotted-wilt-infective thrips. In 1942 all 104 plants of various strains of *Lycopersicon esculentum* and hybrids with Porter's *L. pimpinellifolium* strain were diseased. Of 19 plants of one strain of *L. hirsutum* (P.I. 134,417), all were infected. Twenty-one plants of *L. pimpinellifolium* (Porter's strain) were tested and none showed signs of spotted wilt. Forty-eight plants of 5 strains of *L. peruvianum* (P.I. 126,930, 126,944, 126,946, 128,659 and 129,146) were planted and remained disease-free.

In 1943, 39 plants of 41 from known susceptible lots of *Lycoperiscon esculentum* and hybrids became infected. One of two selections of the German Sugar variety (*L. esculentum*), obtained from W. A. Frazier of the University of Hawaii, showed a high degree of resistance (3 out of 10 plants diseased), while the other selection was susceptible. Ten plants each of two lots of *L. pimpinellifolium,* Porter's strain, and the Fusarium-immune Accession 160 of Bohn and Tucker[2] were planted. Porter's strain remained free from spotted wilt as in previous years while 6 of the 10 plants of Accession 160 were diseased. Of 4 lots of 10 plants each of *L. peruvianum* (P.I. 126,928, 126,944, 128,657 and 128,660) one plant of P.I. 128,660 probably was diseased. The F_1 hybrid of *L. esculentum* and *L. pimpinellifolium,* Ac. 160, was susceptible, as were 5 back-cross lots of *L. esculentum* to the above hybrid. All 69 plants of these hybrids were diseased.

The 2 years' tests confirm the observations of Porter and of Wenholz regarding the resistance of *Lycopersicon pimpinellifolium.* In addition the *Peruvianum* species exhibits a high degree of resistance. The resistance of *L. peruvianum* is difficult to use because this species does not cross readily with the cultivated tomato, although this has been accomplished by an embryo culture technique (to be published elsewhere). The susceptibility

[1] Wenholz, H. Spotted wilt of tomatoes. Breeding for resistance. Hawkesbury Agr. Coll. Jour. **36**: 103. 1939. (Plant Breed. Abs. **10** (1): 65. 1940.)

[2] Bohn, G. W., and C. W. Tucker. Studies on fusarium wilt of the tomato. Immunity in *Lycopersicon pimpinellifolium* Mill. and its inheritance in hybrids. Missouri Agr. Expt. Stat. Res. Bull. 311. 1940.

of one of the two strains of *L. pimpinellifolium* shows that this species is not uniformly resistant. The resistant German Sugar variety of *L. esculentum* may have promise of hybridization. The appearance of the German Sugar suggests that this variety may have had a resistant strain of *L. pimpinellifolium* as an ancestor.—PAUL G. SMITH, Division of Truck Crops, University of California, Davis, California.

Witches' Broom of Beans.—An unreported abnormality of beans was observed at Tucson, Arizona, October 14, 1943, in a victory garden contain-

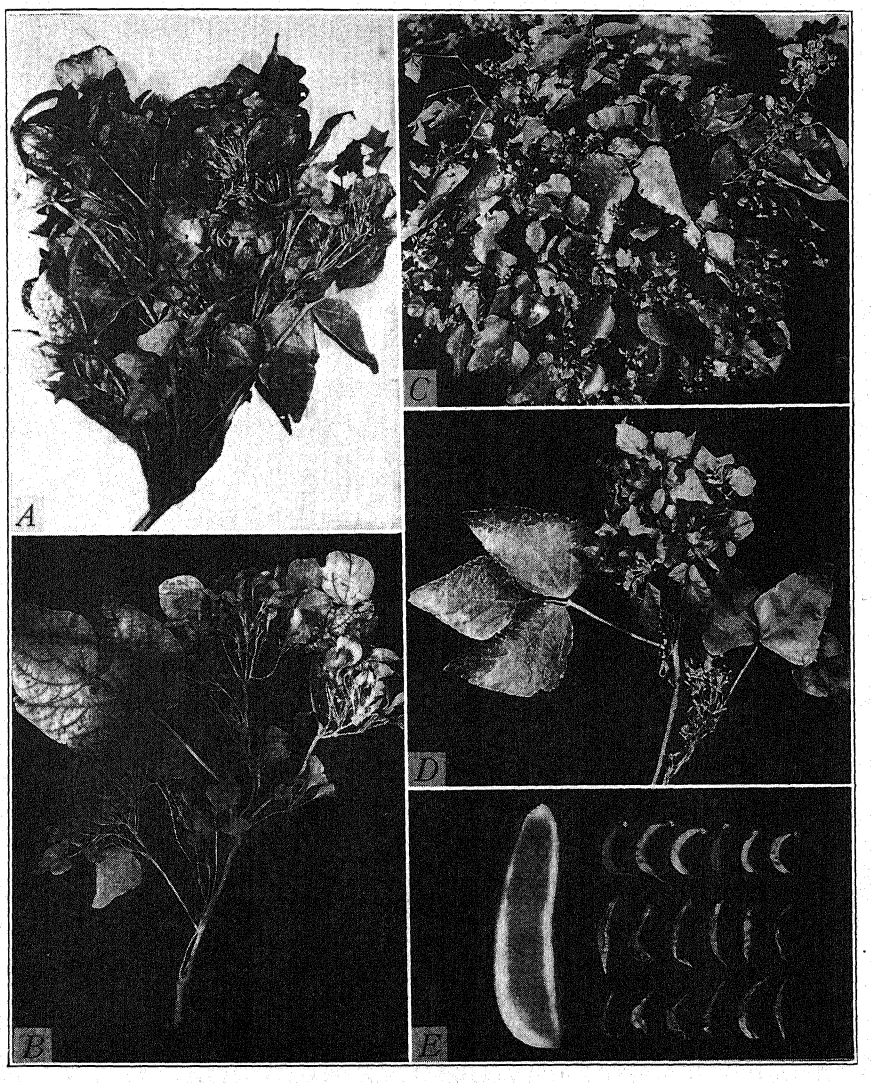


FIG. 1. A. and B. A string-bean plant and a portion thereof showing witches' broom. C and D. A Lima-bean plant and an affected portion. E. One normal and several abnormal pods. The two lower rows of affected pods show considerable wrinkling.

ing six 20-foot rows of string beans. Four plants had an excessive multiplication of branches resulting in symptoms identical to those described for witches' broom. Figure 1, A, illustrates the appearance of an affected plant, while Fig. 1, B, shows a portion of this plant. Pods on the affected parts were small and many were wrinkled. On November 18, 1943, the same abnormality was observed in another Tucson victory garden containing six 16-foot rows of Lima beans. Only one plant (Fig. 1, C) showed symptoms of witches' broom. Figure 1, D, illustrates a portion of the abnormal Lima-bean plant. The affected pods (Fig. 1, E) were $\frac{11}{16}$ to $1\frac{3}{16}$ inches long as compared to a normal pod (Fig. 1, E) measuring $3\frac{11}{16}$ inches. Approximately two-thirds of the affected pods were wrinkled.

Climatic conditions at certain locations in southern Arizona are favorable for spring and fall crops of beans. According to the owners of the victory gardens, their spring beans did not show symptoms of witches' broom. In case of the string beans, the seed for the spring and fall plantings were taken from the same package.—WILLIAM G. HOYMAN, Emergency Plant Disease Prevention Project, Department of Plant Pathology, University of Arizona, Tucson, Arizona.

The Perennial Tree Onion a Carrier of Onion-Yellow-Dwarf Virus.—The perennial tree, top, or topset onion (*Allium cepa* L. var. *viviparum* Metz.) is grown rather widely through the northern tier of States, especially in home gardens, as an early green onion. It is found frequently in gardens in the area of commercial-onion culture in New York State, and is occasionally grown as a market-garden crop in the North, in fields of an acre or more. Newhall[1] has shown that this variety is a factor in overwintering of onion mildew (*Peronospora destructor* (Berk.) Caspary). Circumstantial evidence suggested to H. A. Jones that the tree onion was concerned also in the overwintering of onion-yellow-dwarf virus (*Allium virus 1* (Melhus) Smith, *Marmor cepae* Holmes). Accordingly, 13 plants from New York were supplied to the writers in December, 1942, for the purpose of determining the presence of virus and the symptom expression of onion-yellow-dwarf virus in this variety.

Planted in pots in a greenhouse on December 7, 1942, 4 of 13 plants showed mild yellow streaking at the bases of young leaves on January 1, 1943, but no further confirming symptoms developed on continued growth through November 17, 1943. *Myzus persicae* (Sulz.), fed on leaves of 5 tree onions and transferred to 5 California Early Red seedlings on December 24, 1942, induced strong typical yellow-dwarf symptoms in 1 of 5 test seedlings, recognizable after 14 days and persisting thereafter. On the same date, yellow-dwarf virus from Oregon was inoculated into 5 tree onions by *M. persicae*. No symptoms sufficient for diagnosis appeared up to March 1, 1943, when the same 5 plants were reexposed by *M. persicae* to yellow-dwarf virus out of multiplier onion (*Allium cepa.* var. *solaninum* Alef.), from

[1] Newhall, A. G. The spread of onion mildew by wind-borne conidia of *Peronospora destructor*. Phytopath. 28: 257–269. 1938.

West Virginia. No reliable symptom expression was detected on continued growth to October 20, 1943, when the 5 plants twice exposed to yellow-dwarf by aphids and the 8 plants not experimentally exposed were individually indexed by the carborundum leaf-rubbing method, 5 California Early Red sets serving as test plants for each tree onion inoculation.

These tests showed 6 of 8 noninoculated tree onions infected with yellow-dwarf virus, as well as 5 of 5 that had been inoculated by aphids. In the tests of tree onions that proved positive, from 1 to 5 of the inoculated Early Red sets were infected, while a parallel transfer of yellow-dwarf virus from West Virginia multiplier onions to sets of the same lot by the same method produced 148 infections in 150 sets inoculated. No yellow-dwarf has appeared in control seedlings or sets during 2 seasons' tests. The lower proportion of transfer from the tree onion may indicate that the virus concentration in this tolerant variety is lower than in less tolerant onions, but no evidence was found that the New York virus from tree onion was less virulent than the West Virginia virus from multiplier onion. Two tree onions yielded no virus in these index trials. If repeated indexing reveals no virus in these 2 plants they will serve to produce a virus-free stock of this variety.

The tree onion is essentially a symptomless carrier of yellow-dwarf virus, for no symptoms sufficient for accurate diagnosis were detected during 11 months' growth in the greenhouse in plants thus shown to be infected. The multiplier onion, also grown as a winter onion in gardens, expressed typical yellow-dwarf symptoms in 7 of 12 plants received from West Virginia in 1942. Both varieties of winter onions, the tree and the multiplier, are capable of overwintering the yellow-dwarf virus. In the multiplier variety diagnosis can be made as readily as in commercial varieties, but this is not the case with the tree onion.—PHILIP BRIERLEY and FLOYD F. SMITH, Plant Industry Station, Beltsville, Md.

A Simplified Method of Growing Plants with Roots in Nutrient Vapors.—To facilitate studies[1, 2] of Phytophthora infections and of the toxicity of various concentrations of nitrite and other ions on citrus and avocado roots, the apparatus shown in figure 1 was assembled. This consists of a 12-liter cylindrical glass jar containing 3 liters or less of nutrient, which is recirculated in vapor form over the suspended roots by means of a DeVilbiss atomizer operated with air pressure. To the intake of the atomizer is attached a glass tube, which extends to the bottom of the jar. Clogging of the atomizer by sloughed root material is prevented by filtering the nutrient entering the intake through Pyrex glass wool.

Several obvious advantages of the method are that it permits easy inoculation and examination of the roots, supplies abundant aeration, and readily allows the use of any number of cultures in separate containers of any suit-

[1] Klotz, L. J., and V. P. Sokoloff. The possible relation of injury and death of small roots to decline and collapse of citrus and avocado. Citrus Leaves 23: 1, 2, 3, and 22. 1943.

[2] Sokoloff, V. P., and L. J. Klotz. Distress of citrus trees in association with the temporary presence of nitrite in soil. Citrus Leaves 23: 8–10. 1943.

able size. It also obviates the disadvantage of dilution[3] because the nutrient is recirculated. One needs only to replace occasionally the small amount of solution that escapes as vapor through the holes of the plant support. If desired, the solution level can be maintained automatically with a fount-type of supply vessel. The set-up also lends itself readily to use with the con-

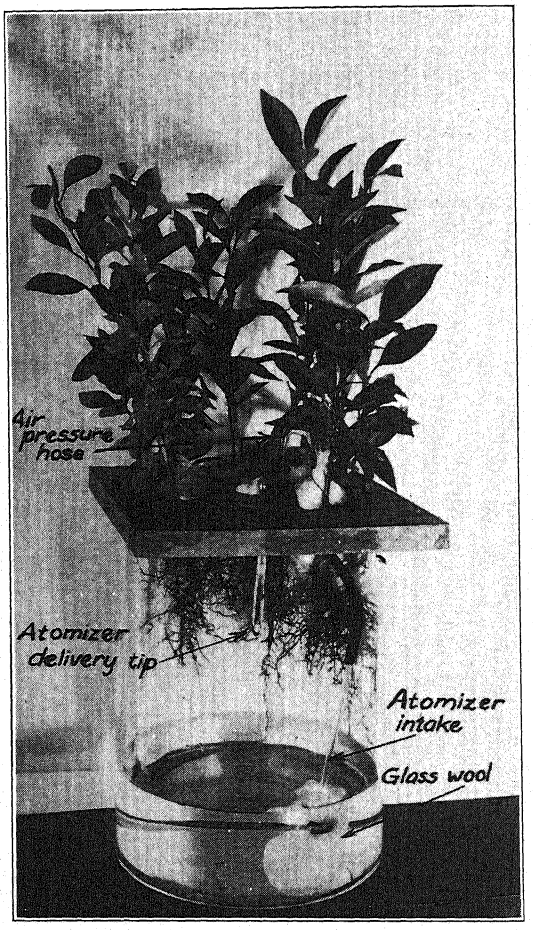


FIG. 1. Apparatus for growing plants with roots in vaporized nutrient.

tinuous flow method[4, 5] of supplying nutrient.—L. J. KLOTZ, Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California.

A Note on the Effects of Splash Injury in Guayule Seedlings.—Guayule seedlings have been grown customarily in nurseries employing overhead

[3] Carter, Walter. A method of growing plants in water vapor to facilitate examination of roots. Phytopath. 32: 623–625. 1942.

[4] Chapman, H. D., and George F. Liebig, Jr. Adaptation and use of automatically operated sand-culture equipment. Jour. Agr. Res. 56: 73–80. 1938.

[5] Eaton, Frank M. Automatically operated sand-culture equipment. Jour. Agr. Res. 53: 433–444. 1936.

irrigation systems. This method of watering sometimes produced an injury resulting in high loss of young seedlings in the cotyledon stage. For want of better name this has been called "splash injury." A brief investigation

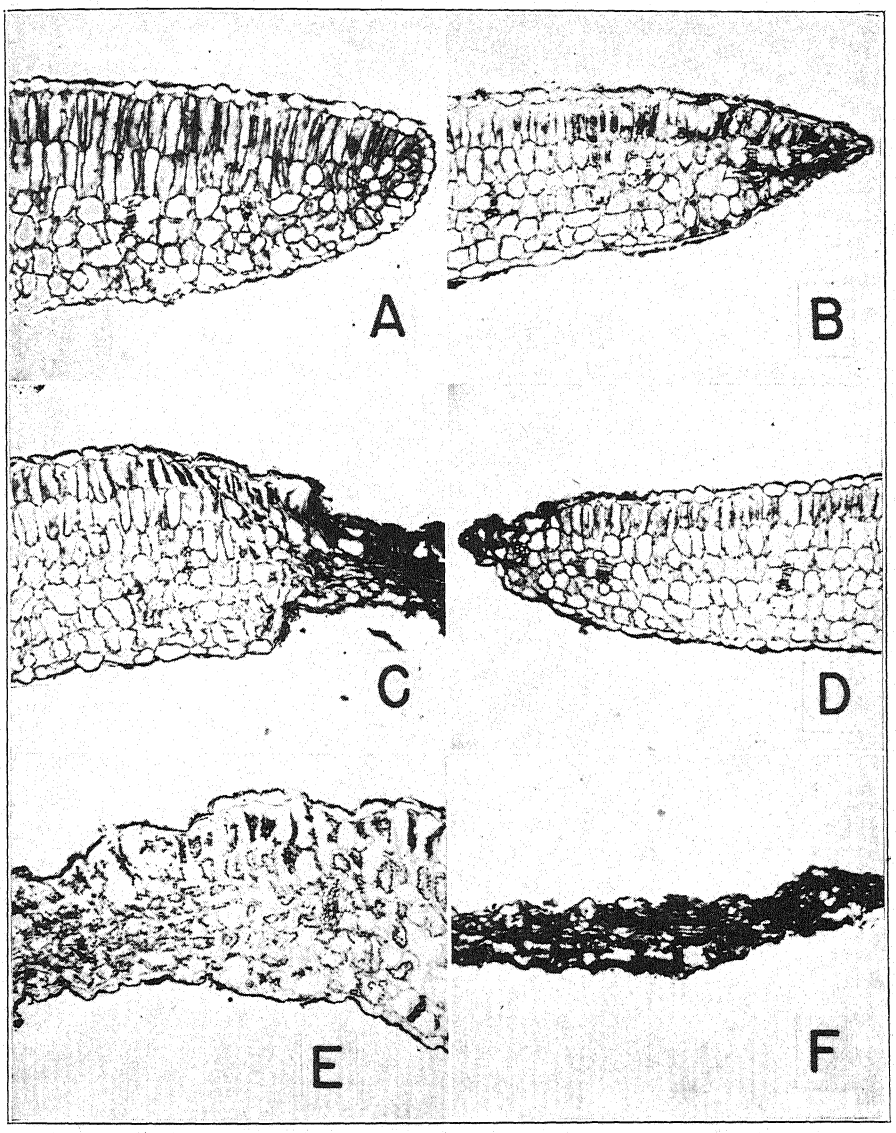


FIG. 1. Sections of splash-injured guayule cotyledons. 1, A. Uninjured cotyledon. 1, B–F. Various degrees of injury. Magnification about 75 ×.

was undertaken to determine (a) the anatomical nature of the injury, and (b) whether microorganisms were present in the injured tissues.

Seedlings showing various degrees of injury were collected from the Alisal Nursery at Salinas, California. They were fixed in form-propriono-

ethanol, dehydrated by means of tertiary butyl alcohol, and stained by the safranine-fast green method of Johansen and the differential fungus-host stains of Ikata and Margolena. The above methods were carried out according to the directions of Johansen's *Plant Microtechnique*. Observations were restricted to sections of cotyledons, as little of the material collected showed injury below the cotyledonary node.

External symptoms of irrigation-splash injury are principally a brown discoloration and withering at the tips and edges of the cotyledons. In severe cases the entire cotyledon, as well as the hypocotyl, are affected.

Various degrees of injury can be seen in the photomicrographs of sections of cotyledons. A portion of an uninjured cotyledon is shown in figure 1, A. Injury usually appeared around the edges of the cotyledon first (Fig. 1, B, D) and then progressed inwards. It sometimes involved the entire cotyledon more or less simultaneously (Fig. 1, E). In either case the final result was the complete collapse of practically all of the parenchyma cells in the cotyledon (Fig. 1, F).

The collapse of cells is preceded by plasmolysis (Fig. 1, E, F) and cytolysis (Fig. 1, B, C, E).

No evidence of microorganisms was observed in the cotyledon sections or in the cotyledonary node and hypocotyl sections. The immediate cause of the injury is, therefore, presumed to be nonpathogenic, brought about in some manner, either by the soil or by water striking the plant or accumulating around it.—FREDRICK T. ADDICOTT, Guayule Research Project, Salinas, California.

A Severe Necrosis Caused by Bean-Mosaic Virus 4 on Beans.—It was shown that bean mosaic virus 4 (*Marmor laesiofaciens* Zaum. and Harter) causes only local infection on some varieties and only systemic infection on others, manifested by a leaf mottling.[1] The varieties susceptible to local infection are immune from systemic infection, and the systemic infected varieties show no local lesions.

A third type of response was recently noted in a strain of the Blue Lake variety. Instead of the local lesions that are produced by the virus on most strains of the variety (Fig. 1, A), small, sometimes indistinct necrotic lesions were observed on the inoculated leaves, mostly in the region of the veins and veinlets (Fig. 1, B). These varied from small, pin-point, dark spots to slightly larger necrotic areas. In some cases only a few lesions were visible, whereas in other cases they were fairly numerous and well-distributed over the entire inoculated leaf. This effect was followed in some instances by a veinal necrosis of varying degrees, depending upon the extent of the infection. Such leaves frequently were killed more rapidly than similar leaves on which only local lesions were produced. A similar type of response was

[1] Zaumeyer, W. J., and L. L. Harter. Two new virus diseases of beans. Jour. Agr. Res. [U. S.] 67: 305–328. 1943.

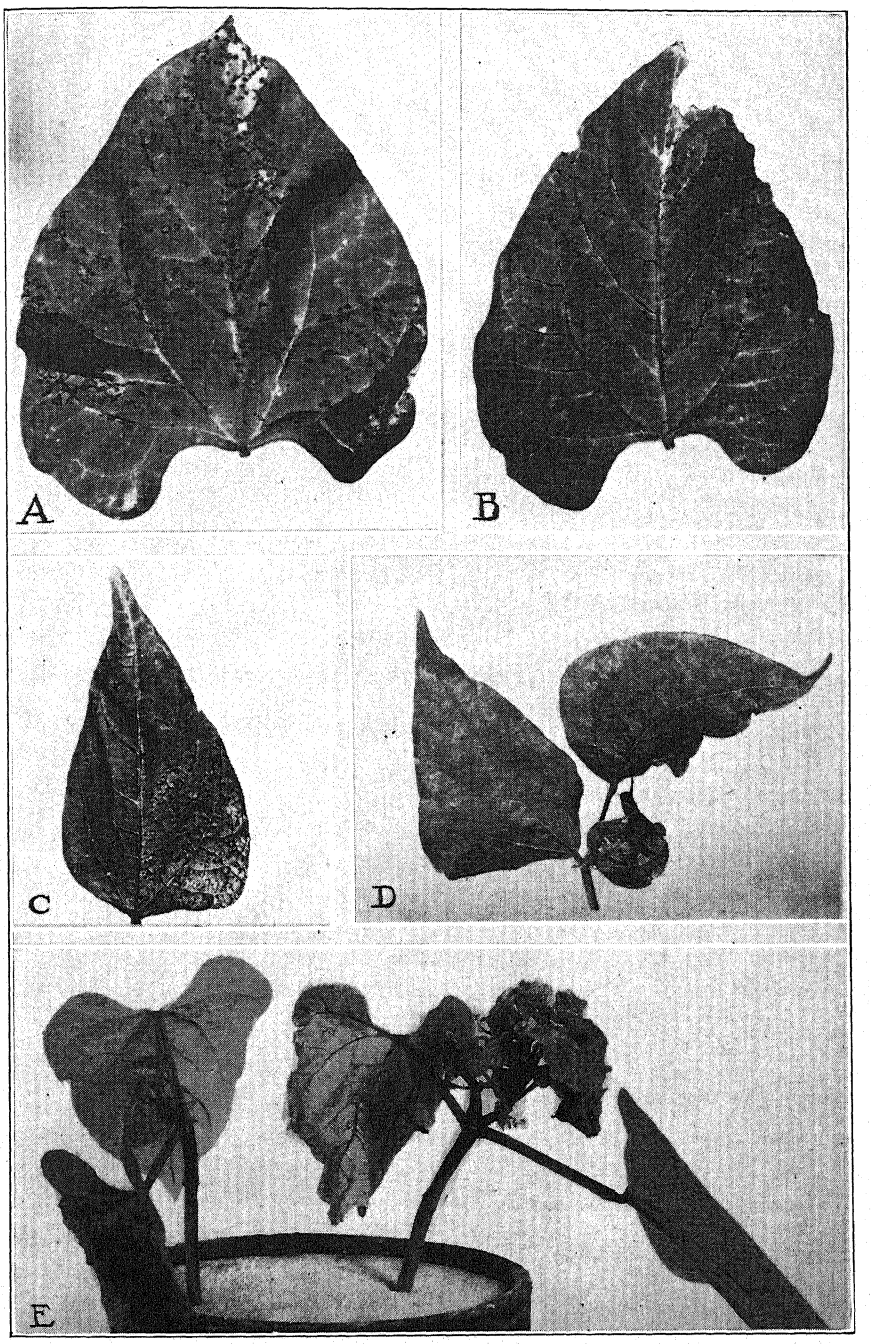


FIG. 1. Variations in symptoms produced by bean mosaic virus 4 on several varieties. A. Local lesions on Pinto. B–E. Effects on a strain of Blue Lake. B, C, necrosis of a primary and a trifoliate leaflet, respectively; D, systemic infection of trifoliate leaflets; E, rosetting of trifoliate leaflets and discoloration of stems and petioles.

reported by Holmes[2] in a few varieties of pepper infected with the ordinary tobacco mosaic virus (*Marmor tabaci* Holmes).

About 10–12 days following these symptoms, irregularly shaped, reddish, necrotic spots appear, most frequently on the upper side of one or more of the trifoliate leaflets (Fig. 1, C). On the underside, the veinlets become reddened, although this effect is frequently noticeable on the upperside as well. Another characteristic symptom is a drooping of the infected leaflets at the pulvini. Although an entire leaflet may show this necrosis, it is common to note the infection on only one side of the midrib (Fig. 1, D). Growth may be almost completely stopped on the necrotic side and thus the leaflet becomes distorted and curls toward the affected portion (Fig. 1, D). In some instances only one leaflet is so affected, whereas in other cases similar symptoms develop on all of the leaflets. Infected leaflets may die, while others may be severely malformed, puckered, and much smaller in size than normal ones (Fig. 1, D).

The stems and petioles of infected plants always show a darkening or a dark, reddish streaking, followed by a slight shrinking.

Another, although less common, type of symptom is characterized by the failure of the young trifoliate leaves to develop, or else when they do develop they become extremely rosetted (Fig. 1, E). They are chlorotic, thickened, and brittle. The internodes are very short, somewhat shrunken, darkened, and extremely brittle. The internal tissues also are necrotic. Such plants frequently remain in this condition for several weeks before dying.

Usually, those plants that showed the greatest amount of necrosis on the inoculated primary leaves were the first to show the systemic necrosis on the trifoliate leaves. Certain inoculated plants that did not show necrotic spots on the primary leaves, manifested a less severe type of necrosis on the trifoliate leaves, which appeared later than on plants developing the necrosis on the primary leaves.—W. J. ZAUMEYER and L. L. HARTER, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Plant Industry Station, Beltsville, Md.

[2] Holmes, F. O. Inheritance of resistance to tobacco mosaic disease in the pepper. Phytopath. 27: 637–642. 1937.

BOOK REVIEW

NAUMOV, N. A. *Rzhavchina Khlebnykh Zlakov v SSSR.* [*Rusts of Cereals in USSR*]. Gosud. Izdat. Kolkh. i Sovkh. Liter., Moscow and Leningrad. 401 pp. 64 figs., 1939. 18r. 50 k. (With chapters on varietal resistance to rusts, selection for rust resistance, and control by agrotechnical methods by E. E. Geshele, and one on damage from rusts by A. A. Shitikova-Rusakova.)

That cereal rusts are no less a problem in the Soviet Union than "in capitalistic countries" is evidenced by such rust losses as 24 per cent (111,000,000 bushels) of the wheat crop and 26 per cent (140,000,000 bushels) of the oats crop in 1933 in but two of the many cereal-producing provinces in Russia. Recognition of the importance of this problem led to the regimentation of Russian phytopathologists and agronomists, beginning some 15 years ago, in an integrated program of developing means of rust prevention. Throughout the past decade the cereal rust literature has been enriched by an increasing influx of practical and theoretical contributions from the Russian workers, many of them original, stimulating, and valuable.

Naumov's "Rusts of Cereals in USSR," the first comprehensive work of this character since that of Yachevski, 35 years ago, comes as a welcome introduction and guide to this new pathological activity. With its background, it is to be expected that the prevailing emphasis of the book is on the practical problem of rust control, yet the theoretical bases of rust etiology and control are not neglected.

Following three chapters dealing with the practical significance of rusts, their developmental cycles, and the significance of heterothallism, there is an extended treatment of the nature and methods of estimation of rust damage, a field in which Naumov, Shevchenko, Rusakov and his coworkers, Ruzinov, and other Russians have made notable, though not well-known contributions.

The following six chapters deal with the nature of rust resistance, varietal susceptibility of cereals to rust, and relation to environment, specialization, and mycology of the cereal rust fungi. *Puccinia graminis, P. triticina, P. glumarum,* and *P. coronifera* are individually treated in detail, while *P. dispersa* and *P. anomala* are touched on briefly. In Russia the most destructive of these is considered to be *Puccinia triticina,* the leaf rust of wheat. On page 33, the author states: "Brown rust (in comparison with stem and stripe rusts) occupies a much wider range in Russia, is better adapted to the climate, appears earlier, develops more quickly, and isn't afraid of the weather," while (p. 204) "as for its economic significance, this species of rust is considered the most important and brings about the most substantial damage to wheat in all the regions where it is met with. In this regard the importance of *P. triticina* is dominant, even over that of *P. graminis,* if we do not count relatively few regions where the economic significance of the two species is locally reversed."

Thirty pages are devoted to epiphytology, another phase in which the Russians, in particular Stepanov, have made noteworthy contributions. Features of cereal rust epiphytology in Russia include: relative unimportance of alternate hosts except locally; long-distance air transport of urediospores, as from Manchuria to Amur province in Siberia; survival of rusts in winter grain fields where the latter are protected by snow cover, forest strips, or features of the microrelief; and local dissemination from winter to spring cereals.

Species of *Thalictrum* play no part in the epiphytology of wheat leaf rust in Russia. The author recounts the work of Brizgalova, who has shown that in Eastern Siberia *Isopyrum fumarioides* is a functioning intermediate host for this rust. On the basis of this work, *Puccinia triticina* is divided into two varieties, var. *isopyri* for the East Siberian form and var. *tritici* for the fungus as known elsewhere in the world. The variety *isopyri* is based on its specialization on *Isopyrum,* its limited geographic range, and the early production and extended period of maturity of its teleutospores. Since the reaction of "*P. triticina* var. *tritici*" on *Isopyrum* has not been tested, the behavior of the teleutospores may be merely a reaction of the typical fungus to Eastern Siberia's rigorous climate, and geographic range alone is not a valid basis for varietal distinctions, it appears to the reviewer that this varietal separation lacks experimental verification.

In control of cereal rusts in Russia, the greatest emphasis is laid on breeding for rust resistance and agrotechnical (cultural) methods. Eradication of alternate hosts is considered to be of local importance only, and sulphur dusting, which has been the subject of study by a number of workers (Shitikova-Rusakova, Rusakov, Zaitseva and Popova, Brizgalova) has not developed to a point of economic significance. Considerable success has been achieved in breeding and selection. The work is carried out under specific instructions of the All-Russian Institute of Plant Husbandry, which includes a formal progression of new strains from breeding nursery to selection nursery, control experiments, preliminary (yield and quality) tests, and, finally, competitive tests at various stations. In the first two nurseries, selection for rust resistance is by observation only, in the last

three, accurate measurements of rust are employed. Breeding and selection have resulted in the production of a number of rust-resistant high-yielding, and adapted strains, including Zarya, and selections from *Triticum erythrospermum*, *ferrugineum*, *cæsium*, and *lutescens* and from the American wheats Marquis, Kanred, Kanred × Fulcaster, and Illini Chief.

Rust control by cultural methods, in addition to practices common in the United States, includes isolation of fields of winter cereals, early spring harrowing of winter cereals, and vernalization of spring-cereal seed (which favors rust-escape by advancing the date of maturity).

Finally, two chapters are devoted to techniques of working with rusts in the laboratory, field, and nursery.

The extensive bibliography includes approximately 600 Russian and 1250 non-Russian titles, and is useful as a check list; however, it is poorly correlated with the text. A high percentage of the works referred to by author and year in the text cannot be found in the bibliography, and many of these represent important bibliographic omissions. An additional difficulty is illustrated by the reference on page 232 to "Gorlenko, 1934." There are 5 papers by Gorlenko in 1934 listed in the bibliography; this problem recurs frequently. There is a 4-page index.

The book is printed on a grade of paper resembling news print, and in a few cases the type is poorly legible; however, the illustrations, a majority of which are from American works, are sufficiently clear. It does not appear possible to purchase the book in the United States, but it may be ordered from Russia through the Four Continent Book Corp., 253 Fifth Ave., New York, N. Y. A translation of sections on epiphytology is available in the libraries of the U. S. Dept. of Agriculture, the University of Minnesota, and Oklahoma Agricultural and Mechanical College.—K. STARR CHESTER, Oklahoma Agricultural and Mechanical College, Stillwater, Okla.

REPORT OF THE 35TH ANNUAL MEETING AND WAR CONFERENCE OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 35th annual meeting and war conference of The American Phytopathological Society was held at The Neil House, Columbus, Ohio, from December 4 to 6, 1943. Fifty-eight papers were accepted by the editorial committee and presented at the meeting. Outstanding was the general interest in all of the sessions and the brisk discussions following presentation of the papers and during special conferences. Approximately 200 members attended the meeting.

The Phytopathologists' dinner was held Sunday evening, December 5, at The Neil House. A delightful and informal program was enjoyed.

The special conferences and discussions included a "fungicide discussion session," the object of which was to discuss the problems of copper and organic fungicides. A round-table conference on the "emergency plant disease program" included progress reports and discussions of needed research and extension based on results of the surveys and more accurate measurements of plant disease prevalence and losses. A report of "insect pest surveys" of the Bureau of Entomology and Plant Quarantine was also given. A symposium of "vegetable seed-borne diseases" was held with discussion centered around the changing scene of vegetable seed production as influenced by the unprecedented demands brought about by lend-lease requirements and Victory gardens. A special session was devoted to the activities of the "war committee." This discussion included the history of the war committee and the present major objectives. This was followed by reports of regional committees and sub-committees of the war committee with considerable discussion from the floor. A conference sponsored by the "extension work and relations committee" included an open-informal discussion on the "possibilities for the plant pathologists to increasingly aid in the war effort," and "how the extension work and relations committee could help the pathologists in 1944." A conference sponsored by the Special Committee "Coordination in cereal and vegetable seed treatment research" included summaries by the various sub-committees plus discussions and suggestions on seed treatments of vegetables, corn, small grains, forage and pasture crops.

The 1943 annual meeting and war conference was considered a very successful meeting and the Society recommended that the "program committee" schedule its 1944 annual meeting at approximately the same time of year.

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R. M. CALDWELL, Treasurer (3 yr. term expires 1946), Purdue University, West Lafayette, Indiana.

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J. G. LEACH (term expires 1944), University of West Virginia, Morgantown, West Virginia.
J. C. WALKER (term expires 1944), University of Wisconsin, Madison, Wisconsin.
H. P. BARSS (Potomac Division), Office of Experiment Stations, U.S.D.A., Washington, D. C.
O. C. BOYD (New England Division), Massachusetts State College, Amherst, Massachusetts.
A. G. PLAKIDAS (Southern Division), Louisiana State University, Baton Rouge, Louisiana.
E. E. WILSON (Pacific Division), University Farm, Davis, California.

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A.A.A.S. Council. L. M. HUTCHINS, J. G. LEACH.
Division of Biology and Agriculture, National Research Council. J. C. WALKER (3 yr. term expires June 30, 1946).
Board of Editors, American Journal of Botany. A. A. DUNLAP (3 yr. term expires 1946).

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REPORTS OF OFFICERS, REPRESENTATIVES AND COMMITTEES FOR 1943

Report of the Secretary. At the time of our Council Meeting, February 12, 1943, the membership was 1086. It now totals (December 6, 1943) 1060. This makes a net loss of 26 members. During the period from February 12 to December 6, 1943, 30 individuals applied for membership. By action of the Society all applicants were elected on December 6.

Eight former members have been reinstated, and the Society lost a total of 64 members: 11 by resignation, 3 by death, and 50 suspended for nonpayment of dues. Of the full membership, 155 are paid-up life members, and one was paying $10.00 a year toward life membership until his payments were interrupted by conditions abroad.

The Secretary calls attention to the fact that suspended members may be reinstated by notifying the Secretary and paying dues for the current year. Former members should be advised of this and urged to seek reinstatement, if their professional work is directly related to plant diseases and their control.

The Clearing Agency of the Society was continued in 1943. There are 71 applications in the active file—27 of these new during the year. One hundred three applications of 51 pathologists were sent to 14 employers, who made request for applications. Several applicants were hired by contacts made through our Clearing Agency.

Report of the Treasurer. Statement of accounts for the year ending November 30, 1943.

Receipts:

Balance from 1942			$1964.28
Annual dues:			
1939–1940	$ 10.00		
1941	10.00		
1942	46.42		
1943	2766.41		
1944	1926.90		
1945	1.00	$4760.73	
Voluntary dues		5.00	
Donation for foreign subscription		5.00	
30-Year Index		6.25	
Sales		1.00	
Reimbursement for checks returned by bank		9.50	
Total receipts			4787.48
			$6751.76

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY:			
1939–1940	$ 8.00		
1941	8.00		
1942	36.67		
1943	3554.16	$3606.83	
Transferred to PHYTOPATHOLOGY for:			
Voluntary dues	5.00		
Foreign subscription donation	5.00		
Sales	1.00		
Index	6.25	17.25	
Transferred to Sinking Fund		15.00	
Secretarial work and expenses of office of Secretary		215.15	
Secretarial work for Treasurer		288.00	
Printing		208.73	
Stamps and envelopes		63.39	
Express		0.63	
Demonstration program		6.96	
Bank service charge		13.54	
Checks returned by bank		20.00	
Total expenditures			4455.48
Balance on hand			2296.28
			$6751.76

Sinking Fund. The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, was obtained by deposit of payments from patron memberships and deductions from life-sustaining membership instalments. All life-sustaining members, with the exception of one in Italy, have paid in full. There has been no change in the amount of the fund during the past year, the total remaining at $9676.00. It is invested as follows:

First mortgage note deposited with McLachlen Banking Corporation for collection ($500.00 at 4½%)	$ 500.00
United States Savings Bond, Series G, 2½%	1000.00
Invested with the following building and loan associations:	
Arlington and Fairfax Bldg. and Loan Association, 4%	1000.00
Columbia Permanent Building Association, 4%	520.20
District Building and Loan Association, 3½%	1556.78
National Permanent Building Association, 4½%	2091.00
Northwestern Federal Savings and Loan Association, 3½%	2000.00
Perpetual Building Association, 4%	1040.40
Prudential Building Association, 3½% (accrued interest, $14.71)	190.71
	$9899.09
Less interest due PHYTOPATHOLOGY	223.09
	$9676.00

The Lyman Memorial Fund, obtained from voluntary contributions, now totals $3175.32. The whole amount is invested with the Brookland Building Association, at 3½%. The account for 1943 follows:

Balance on hand, December 1, 1942	$3132.57
Dividends, December 31, 1942, and June 30, 1943	110.51
Contributions from members	37.00
Sale of Erwin F. Smith Memoir	5.75
	$3285.83
Less interest due PHYTOPATHOLOGY	110.51
	$3175.32

Additional Endowment. During the past year the Committee on Legacies and Donations has secured as of this date (Dec. 1, 1943) and turned over to the Treasurer, United States Savings Bonds, Series F, having maturity value of $725, and stamps and cash totaling $6.50.

Report of the Business Manager. At the close of 1942 there were 416 nonmember subscribers, including 6 complimentary. In 1943 there were 7 cancellations and 48 suspensions for nonpayment, a loss of 55. As there were 70 new or restored subscriptions, a slight net gain of 15 resulted, bringing the total subscription list to 431. The gain from 200 to 240 in the domestic list was due in large part to the fact that 24 subscriptions for eventual shipment to U. S. S. R. were ordered sent to their agent in New York. There was also an order for 7 subscriptions to be sent to New York, for storage until the numbers can be sent to The Netherlands. There were a few other orders for subscriptions for future shipment to foreign countries. Again the American Library Association has ordered and paid for 45 sets of Vol. 33, to be stored with our printers for eventual shipment to foreign countries as they may designate. These 45 are not included in the total subscriptions above, but are listed as sales.

Statement of accounts for the year ending November 30, 1943.

Receipts:

Balance from 1942		$ 7010.09
Subscriptions:		
1937	$ 6.50	
1939–1941 incl.	19.50	
1942	192.85	
1943	2214.50	
1944	482.35	
1945	17.00	$2932.70
Member subscriptions:		
1939–1940	8.00	
1941	8.00	
1942	36.67	
1943	3554.16	3606.83
Sales of back numbers (including orders for current numbers reserved for American Library Association)		999.70
Advertising:		
1942	289.41	
1943	834.33	1123.74
30-Year Index		227.60
Interest on Sinking Fund:		
First mortgages	88.40	
Building and Loan*	110.10	198.50
Interest on current funds		158.88
Grant from Rockefeller Institute		600.00
Allowance on reprints		466.93
From authors for excess illustrations		221.54
Part payment for article in November, 1942, issue		50.00
First mortgage note paid in full		1000.00
Total receipts		11586.42
		$18596.51

* $223.09 accrued interest left on deposit with Building and Loan Associations, $110.51 with Lyman Fund.

Expenditures:

Printing, distributing, and storing PHYTOPATHOLOGY:			
Vol. XXXII, No. 11	$900.78		
No. 12 and Index	754.54		
Vol. XXXIII, No. 1	734.07		
No. 2	726.77		
No. 3	704.75		
No. 4	673.58		
No. 5	721.47		
No. 6	836.45		
No. 7	857.57		
No. 8	904.29		
No. 9	719.24		
No. 10	996.70	$9530.21	
Postage, PHYTOPATHOLOGY	398.57	$9928.78	
Secretarial work and office expenses, Editor in Chief		352.25	
Secretarial work for Business Manager		325.00	
Secretarial work for Advertising Manager		112.87	
Stamps and envelopes		36.58	
Supplies and furniture		41.20	
Printing		25.75	
Postage, 30-Year Index (part)		8.25	
Insurance on back volumes		3.04	
Reinvestment of funds, Sinking Fund		1000.00	
Refund, subscription		6.00	
Check returned by bank		6.50	
Total expenditures			$11846.22
Balance on hand:			
Checking account		2091.41	
Northwestern Federal Savings and Loan		4658.88	6750.29
			$18596.51

The 30-Year Index. In September, 1941, an edition of 1000 copies was printed. From a running inventory of orders we have recorded shipment of 672 copies, and 14 copies are being held for future shipment to members and subscribers in occupied or enemy countries. Allowing for copies damaged or lost in transit, this should leave approximately 300 copies on hand at the printers'. A summary of receipts and expenses, included in financial reports for 1941, 1942, and 1943, follows:

Receipts:		
Sales of index, April, 1941, to November, 1943, inclusive		$3271.38
Expenses:		
Honoraria to editor and collaborators		762.50
Printing and distribution		2084.50
Other expenses		364.80
		$3211.80
Receipts in excess of expenditures		59.58

Figures given do not take into consideration accounts payable or uncollectable accounts.

Report of the Auditing Committee, as of December 1, 1943. We have examined the books and find them correct, with entries completely supported by vouchers. We wish to congratulate the Business Manager and Mrs. Meier on the clear presentation they have given of the financial transactions.

SEARS P. DOOLITTLE, *Chairman*
H. A. RODENHISER

Report of the Advertising Manager for 1943. During the year 1943 there were 108 revenue-producing advertisements carried in PHYTOPATHOLOGY. These advertisements occupied 71 pages and consisted of 43 full-page, 47 half-page, and 18 quarter-page insertions. Seventeen commercial companies used PHYTOPATHOLOGY as an advertising medium in 1943. Gross advertising contracts totalled $1265.00. Net profit to the journal for the year, after cost of printing, secretarial expenses, agency commissions and discounts are deducted, will be approximately $750. The journal would be printed regardless of advertising space contracted for in each issue, hence the profit figure given above is the difference between the cash receipts from advertising and the estimated additional cost.

In addition to the above revenue-producing insertions, 22 nonrevenue-producing advertisements were carried. These consisted of 4 full-page, 10 half-page, and 8 quarter-page insertions relative to Phytopathological Classics, the Society Clearing Agency, the 30-year Index, and Biographical Memoir of Erwin F. Smith. Costs of inserting the nonrevenue-producing advertisements were included in determining the net profit from advertising.

The management has been considerably chagrined by the lack of cooperation and consideration shown by members of the Society directed towards securing advertisements. From the above figures it can readily be seen that advertising contracts could, with member cooperation, be great enough to aid materially in offsetting the expenses of publishing the journal. A plea for cooperation was made at the Thirty-Second Annual Meeting, but during 1943, only one lead for prospective advertising was referred to the Advertising Manager. Certainly the members of the Society are capable of greater coordinated effort and the management of PHYTOPATHOLOGY earnestly asks for better support in 1944.

S. L. HOPPERSTEAD,
Advertising Manager

Report of the Editor in Chief. Volume 33 of PHYTOPATHOLOGY, exclusive of its index, contains 1218 pages of printed matter and illustrations, classified as follows: One hundred eighteen articles, 49 notes, a report of the Columbus meeting of the A. P. S. Council in February, 2 reports of the War Committee, 3 reports of the Committee on Standardization of Fungicidal Tests, 2 book reviews, 139 abstracts, 250 text figures and 1 plate.

From Dec. 10, 1942, to Nov. 30, 1943, a total of 165 manuscripts of articles, notes, book reviews, and reports were submitted for publication. Five manuscripts were either recalled by their authors or returned to them as unsuitable for publication in our Journal. There are now (Nov. 30) in press 34 articles and 53 abstracts. Other manuscripts on hand, as of that date, total 578 typewritten pages. It is estimated that there are on hand and accepted a sufficient number of manuscripts to fill about 660 printed journal pages. Of these manuscripts a considerable number, amounting to 102 pages, are in process of revision by their contributors.

The quality and importance of PHYTOPATHOLOGY as a research journal depend in very large measure upon the character and scientific excellence of the papers we contribute. That there has been an evident improvement over the years none can deny; still, there is room for improvement. Much of the delay in publication of approximately 20 per cent of all manuscripts ultimately accepted by the editors of our Journal is directly chargeable to the contributors. This becomes clear when we realize that final acceptability of such manuscripts has necessitated in some instances as many as three and four round trips between author and editor.

By increasing the number of pages per journal number we have been able to shorten the interval between date of acceptance and date of publication from 9.2 months in June, 1943, to 7.5 in November. The outlook for a further shortening is promising, partly because of the temporary policy of increasing the number of pages per number and in part because of the rather marked decline in receipt of manuscripts. We have thus far been fortunate in being able to receive our journal with remarkable regularity, and singularly free from the annoyances and embarrassments attendant upon such labor, material, and other conditions as are peculiar to such an extraordinary upheaval as the world is now experiencing.

H. B. HUMPHREY

Report of the Manager of Phytopathological Classics for the year 1943. I beg to submit herewith the annual report of my stewardship as Manager of Phytopathological Classics.

Report for the fiscal year beginning December 1, 1942, and ending December 1, 1943

Classics No. 1:	On hand 12–1–42	34	
	Sold during year	11	
	On hand 12–1–43		23
Classics No. 2:	On hand 12–1–42	234	
	Sold during year	11	
	On hand 12–1–43		223
Classics No. 3:	On hand 12–1–42	326	
	Sold during year	12	
	On hand 12–1–43		314

Classics No. 4:	On hand 12–1–42	387	
	Sold during year	13	
	On hand 12–1–43		374
Classics No. 5:	On hand 12–1–42	626	
	Sold during year	12	
	On hand 12–1–43		614
Classics No. 6:	On hand 12–1–42	712	
	Sold during year	16	
	On hand 12–1–43		696
Classics No. 7:	On hand 12–1–42	762	
	Sold during year	44	
	On hand 12–1–43		718

Receipts:

Cash balance on hand 12–1–42	$358.72	
Receipts during year	91.10	
Total		$449.82

Expenditures:

Postage	$ 7.25	
Journal	.29	
Total expenditures		$ 7.54
Balance on hand December 1, 1943		$442.28
Due on accounts 12–1–43		17.25

H. H. WHETZEL

Report for 1943 of the Representatives on the National Research Council. Energetic activity characterized the year's endeavor of the Division of Biology and Agriculture of the National Research Council to serve the Nation's war effort and to meet present and future problems growing out of the world conflict. Most of the Division's many committees were functioning with accelerated momentum on wartime objectives. A number of important conferences were called during the year to consider various problems growing out of the war. The following necessarily limited report covers only partially the Division's work.

At the Annual Meeting held April 10, 1943, in Washington, D. C., on nomination from the Society, J. C. Walker was elected to succeed Howard P. Barss as representative for three years beginning July 1, 1943. Robert F. Griggs and A. L. Maynard were reelected Chairman and Vice Chairman of the Division. R. E. Coker, A. C. Redfield, and Howard P. Barss were elected to the Executive Committee. S. E. A. McCallan of this Society was nominated to serve on the Board of Governors of the Crop Protection Institute to succeed H. T. Cook, now in overseas service with the Navy.

At this meeting major consideration was given to the crippling effect of current Selective Service procedures on technological wartime services in the fields of biology and agriculture. No clear solution of this serious problem was presented but the Division recommended the establishment of a committee to work with the proper war agencies on critical manpower shortages in these fields. J. S. Nicholas and J. G. Leach were later charged with this responsibility. It also recommended the establishment of a committee to work with appropriate war agencies in organizing effective research on problems arising in the war effort which involve the biological sciences. Attempts made later to find means to set up such a biological research committee were not successful. A new general National Research Council Committee on Scientific Personnel was, however, set up. The members are: H. A. Barton, Chairman, L. P. Eisenhart, R. F. Griggs, W. W. Rubey, and H. Marston Morse. Funds contributed by scientific organizations provide the committee with a working staff under Dean H. L. Dodge.

An interesting discussion, led by E. J. Kraus, dealt with the need for more adequate basic training as preparation for agricultural research. This grew out of a recommendation of the N.R.C. Fellowship Board that the Division set up a committee to encourage better training for research in agriculture. The committee personnel is: Richard Bradfield, Chairman, J. H. Gourley, C. B. Hutchison, J. L. Lush, and E. C. Stakman.

The Division was pleased to announce completion of projects, which included E. D. Merrill's manual of food plants and poisonous wild plants for the Pacific Islands, the manual of plants of the Central American-Caribbean region by P. C. Standley and B. E. Dahlgren, and Standley's manual of plants for the Arctic. Publication was in the hands of the War Department. The need for these manuals is evidenced by the fact that no less than 19 separate agencies of the armed forces, mostly without adequate technical help, had started efforts along such lines.

The Crop Protection Committee, through its acting chairman, J. G. Leach, reported on its joint meeting with the War Committee of the Society at Columbus, Ohio, in February. The ideas exchanged were helpful to entomologists and plant pathologists alike. A fine spirit of cooperation between them was noted. A manpower survey was reported under way by both groups. A profitable symposium, led by C. I. Bliss, on coordinated dosage experiments to determine the best way to economize on critical fungicides and insecticides was sponsored by the Committee. The Committee reported efforts toward a strengthening of Federal crop pest and disease information services. The need was presented for a study of the influence of wartime cropping changes over wide areas on the insect and disease situation.

Under the chairmanship of E. C. Stakman, the Crop Protection Committee met again in August with the Society's War Committee and Upper Mississippi Valley Group at Purdue University. The encouraging insecticide and fungicide supply was reviewed. Resolutions were presented for wartime intensification of barberry and buckthorn eradication and for all possible activity against further spread of the "Dutch" elm disease. J. S. Houser was appointed to succeed the late W. P. Flint on the Committee.

At its April meeting the Division reaffirmed its belief that "Societies in the field of science and technology represented in the Division of Biology and Agriculture of N.R.C. should plan to hold their annual meeting; that such meetings preferably not be held during Christmas holidays; that at least one session be devoted to the discussion of war problems."

D. M. Whitaker of Stanford University served the Division as Executive Secretary from February to the autumn of 1943, devoting much energy to the difficult and still unsolved problems of manpower and of mobilizing biological science for the war effort.

Throughout the year attention was given by the Division, under the chairman's leadership, to the question of how scientists in all fields of biology and agriculture can best marshall their combined strength to meet the Nation's wartime and post-war needs. Work on this problem of organization is still in progress.

The National Research Council Food and Nutrition Board, F. G. Boudreau, Chairman, continued its important activities. The Board's many committees carried out work on National wartime nutritional and food enrichment standards and on many other important scientific undertakings in the food and nutrition field.

The Committee on Animal Nutrition, under L. A. Maynard's chairmanship, with its subcommittees, was active and working closely with the Food and Nutrition Board. A Committee on Animal Health was appointed with G. H. Hart as chairman.

The Committee on Instruction in the Biological Sciences met in Cincinnati in January, 1943, and reported in favor of separate introductory courses in botany and zoology with some field work in each. E. L. Stover is Chairman.

J. C. WALKER
HOWARD P. BARSS

November 15, 1943.

Report of Committee on Donations and Legacies. During 1943 an attempt was made to interest members of the Society in investing sums of which they were capable in War Bonds and Stamps in the name of the A.P.S. Appeal letters were forwarded to all members on February 10, 1943, and May 27, 1943. The response has not been gratifying. From 23 members, bonds and stamps having a cash value of $543.00 have been received. This represents an average of approximately 50¢ per member. This is not a good record. The record on percentage of contributors is even worse; only 2 tenths of 1%. Surely, this is not indicative of the best that our profession should voluntarily produce.

The committee is gratified to have added this small amount to our endowment funds during the year, but sincerely disappointed over the response of the membership.

We recommend that a further appeal be made in the coming year, urging members of A.P.S. to support the Endowment of "Phytopathology" through the agency of War Bonds and Stamps.

E. C. STAKMAN, J. G. BROWN, N. J. GIDDINGS, NEIL STEVENS,
RICHARD P. WHITE, Chairman

Report of the Extension Work and Relations Committee. The Extension Work and Relations Committee in 1943 sponsored an open, informal discussion session at the December meeting of the Society. This session had for its objectives aiding members in plan-

ning extension projects for 1944, and also planning a program of work for the committee.

In addition, through its War Emergency Subcommittee, the following activities have been carried on:

(1) A Kodachrome transparency exchange has resulted in the preparation and distribution of duplicate sets totaling 3,818 slides on vegetable diseases and 1,197 slides on fruit diseases. It is hoped to continue this line of work on other crops.

(2) A manual of extension methods in plant pathology was prepared by a group of pathologists under the editorship of Dr. Haskell, and distributed to plant pathologists and heads of extension work throughout the United States and Canada.

(3) A manual of disease control practices has been prepared and is awaiting a means of publication.

(4) Since the last published report of this committee a survey of extension work in plant pathology throughout the United States shows that 16 of the 37 stations filling in the questionnaires have no organized extension projects in plant pathology, while 21 have. Results from this questionnaire were given at the 1942 summer meeting at Toledo.

At the summer meeting of the War Emergency Committee at Lafayette, the committee sponsored a movement to revive the EXTENSION PATHOLOGIST.

O. D. BURKE

Report of the Necrology Committee. During 1943, there were three deaths within our membership as follows:

ANDRÉS R. LÓPEZ, February 4.
ISAAC MCKINNEY LEWIS, March 12.
H. E. PARSON, November 18.

A. G. JOHNSON, Chairman
M. B. WAITE

Report of the Public Relations Committee for 1943. This committee has continued to function along lines presented in the report for last year. The efforts expended by the individual members have been greater than during the previous year with less apparent success. In some instances excellent cooperation with editors and publishers in agricultural journals and in the newspaper field has resulted in the use of material on plant pathology. Generally, however, the response has been poor, possibly because of the lack of proper preparation of the articles, possibly because this type of material has been crowded out entirely by war news and appeals for production efforts. The Committee solicits the services of members who have ability in this publicity field.

All numbers of the journal have been promptly handled, and the same responsibility can be expected for the future. Any suggestions or constructive criticisms aimed at the improvement of the projected work is requested and appreciated.

The Committee wishes to thank all who have contributed toward its functioning during the year.

L. M. BLANK, O. C. BOYD, K. S. CHESTER, J. J. CHRISTENSEN, C. T. GREGORY, J. H. JENSEN, F. P. MCWHORTER, A. G. NEWHALL, J. A. PINCKARD, L. SHAW, G. H. STARR, C. E. YARWOOD, G. F. WEBER, Chairman

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The plant quarantine aspect of the outbreak of the so-called golden nematode (*Heterodera rostochiensis* Woll.) in Long Island, N. Y., in 1941 is of outstanding interest. This soil parasite seems to be spreading locally with some persistence and presents a quarantine problem which ought to be met promptly and studied with care from every angle.

As a matter of possible interest, this committee has prepared a summary of the various proposals and recommendations made by the Society in the quarantine and regulatory field during the past 15 years. A copy of this review is being filed with the Secretary and further copies can be obtained by members who may have an interest in the subject. It is suggested that some report on the progress or status of these items be presented to the Society at its next meeting. Your committee suggests that there be added to this existing list of subjects to which the Society has given and will continue to give profitable attention the following:

(1) Bacterial diseases as a foreign plant quarantine problem.
(2) The advisabiilty of making arrangements to have certain imported field and vegetable seeds treated before distribution here, as a protective measure parallel with and complementary to the domestic seed treatment measures advocated by the Society's War Board.
(3) A discussion of the value of certification in the country of origin in protecting this country against foreign disease introduction.

This Society has on several occasions expressed a deep interest in proposals for potato wart eradication from the limited areas in the three states in which it is known to occur. Intimately connected with this proposal, however, is the need for determining the correct

national policy which ought to be adopted toward the importation of foreign potatoes, either from the standpoint of potato wart alone, or in a larger way to include consideration of all foreign potato diseases and insects with a view to securing as high a degree of protection as possible for one of this country's basic food crops. In view of the probability that post-war trade resumption may involve demands for increased foreign potato importation it is important that our foreign plant quarantine policy towards potatoes be established now on a sound basis as an adequate guide to future quarantine administration. It is believed that the Society can contribute usefully to the country's welfare and render a definite service to the federal administration by giving its best thought to both aspects of the wart problem, and it is recommended that a committee be constituted either by suitably enlarging the present committee or by the appointment of a separate committee, as the Society may desire, to study the subject and report with recommendations at an early date. It may be assumed that all available information will be placed at the disposal of such a committee.

C. R. ORTON, R. P. WHITE, E. C. STAKMAN, Chairman

Report of the Committee on Biological Abstracts and the Union of American Biological Societies. BIOLOGICAL ABSTRACTS has successfully passed another milestone in its difficult wartime course. Though still a chronic issue, the financial situation now takes second place before the problem of personnel maintenance. Fortunately the editors, Dr. John E. Flynn and Dr. Jean MacCreight, as well as H. I. Anderson, business manager, continue to stand by the ship at a considerable personal sacrifice. To them the biological fraternity is deeply indebted. The resignation of several key members of the bibliographic and clerical staff, however, has had its adverse effect, particularly because of replacement difficulties. As a result, the central office staff has shrunken to four less than the number in 1940. Furthermore, the resignation of collaborators entering the armed forces, frequent changes of address, and the preoccupation of so many of the trustees, section editors and collaborators with pressing wartime duties have likewise added very appreciably to the difficulties encountered.

Nevertheless, Biological Abstracts has continued full speed ahead. The 1943 volume will contain 25,947 abstracts—2500 over 1942 in spite of evident shrinkage in many important journals. Phytopathologists will be interested in the fact that abstracts in the plant disease field number 956 in 1943—over 11 per cent more than the year before. The current increase for the complete edition is mainly accounted for by the extension in coverage to some 1800 periodicals—about 100 more than in 1942, largely because those of the Axis and German-occupied Europe are now becoming available through microfilm.

The separately published subject-matter sections continue to increase in popularity. The new Section G—Abstracts of Food and Nutrition Research is already firmly established. Subscriptions to sections have increased 24 per cent and to the complete edition 10 per cent, the total reaching an all-time high of 3634 paid subscriptions, or 19 per cent above 1942. Subscriptions to Section D—Abstracts of Plant Science have reached 455 (382 domestic, 73 foreign). By the end of October, some 13 new subscriptions had been received from members of the Society as a result of circularization in late summer. With respect to the plant disease material in Biological Abstracts, the Editor-in-Chief has this to say: "In the field of phytopathology, it is a pleasure to acknowledge that whatever success we may have had in the publication of scholarly abstracts and indexes is mainly due to the fine and careful work of its editor, Doctor Freeman Weiss. Doctor Weiss has labored with rare devotion, and the scholarship such as one sees all too infrequently. He deserves the Society's thanks for his services as editor given wholly without compensation."

Biological Abstracts of course still proceeds under considerable financial difficulty. The support of American biologists, as reflected in abstracting done and in willingness to work for the success of the whole enterprise, continues to be outstanding and their backing as expressed in financial terms is likewise excellent and on the increase. Nonetheless the income from subscriptions falls considerably short of what is required to produce Biological Abstracts. In 1942, $5,436 had to be withdrawn from the reserve. In 1943, thanks to contributions from biological societies estimated at over $3,500, and to donations from some 50 corporations already totaling nearly $9,000, draft on the reserve may perhaps be avoided. It should be emphasized, however, that grants from industrial concerns are not necessarily recurrent. It is therefore to increased subscription income that the trustees must look, in the main, for the funds that will put the enterprise on a sound sustaining basis. Not only the trustees and editors of Biological Abstracts, but the whole biological fraternity, owe gratitude to the biological societies and industries whose contributions are helping to maintain the service through this difficult period.

As they compare what has been accomplished with the needs for building up an abstracting and fact-gathering service that would more completely serve the nation's interests and the requirements of its biologists, the editors of Biological Abstracts recognize that present achievement is still far from the goals they have in mind. Every pos-

sible effort, however, is being put forth to obtain more and more of the European publications and to get them promptly and expertly abstracted, although this must now be done largely from microfilm copy. Indexing on the scale required for Biological Abstracts is a task of enormous complexity and difficulty—a job for experts in each subject matter field. Considerable progress has been made during the year in developing a staff of indexers and in working out techniques that should enable publication of future indexes at progressively earlier dates. The trustees and editors feel a great responsibility for maintaining the continuity of this important service through the present world catastrophe and beyond. In proportion as the backing is forthcoming can its performance be enlarged and improved.

FREDERICK V. RAND, Chairman

Nov. 6, 1943

Resolutions of the Committee on Resolutions. RESOLVED that The American Phytopathological Society convey to the management and staff of The Neil House, and especially to Mr. V. C. Murphy and Miss Kathryn Weitzel, its appreciation for the courteous service extended to our members and for cooperation in providing our officers and committees with unusually commodious quarters for conducting business and scientific programs.

RESOLVED that the Society express to the Committee on Local Arrangements, Dr. W. G. Stover, C. W. Ellett, R. U. Swingle and H. E. Reed, our gratitude for their fine contribution to the success of the meeting.

RESOLVED that this Society commend Dr. H. A. Edson, our Treasurer and Business Manager, for his very efficient administration of the affairs of the Society, extending through nearly a decade, and for his tireless efforts and his keen business insight reflected in our Journal, and the Society's investments, which have flourished as never before in the history of the Society.

RESOLVED that the Society commend Mrs. Agnes E. Meier, Assistant Business Manager, for her very efficient work in serving the best interests of the members of the Society.

RESOLVED that the Society express their gratitude to the Botany Department of the Ohio State University for very kindly placing at the Society's disposal projection apparatus, its good offices, laboratories and personnel. To the Horticultural Department our appreciation for the beautiful bouquet of flowers that graced our banquet table.

RESOLVED that the Society express its appreciation to Professor E. S. Thomas for his very interesting and educational illustrated lecture on "Outdoor Ohio in Natural Color."

RESOLVED that this Society on behalf of its members express to the officers and members of committees our sincere appreciation for the efforts they have made to promote the interests of the Society throughout the year, and to make this annual meeting instructive and enjoyable.

Respectfully submitted,

CHARLES CHUPP
I. E. MELHUS
C. M. TUCKER, Chairman

ACTION TAKEN BY THE COUNCIL AND SOCIETY AT THE 1943 ANNUAL MEETING, AND THROUGH MARCH 1, 1944

Elections and Appointments. The election committee opened and counted the ballots and the results were announced to the Society at the banquet. The names of those elected and appointed appear earlier in this report in the list of officers, representatives, and committees.

Thirty applicants were elected to membership in The American Phytopathological Society.

Following the 1943 annual meeting, the Editor-in-Chief of PHYTOPATHOLOGY, Dr. H. B. Humphrey, submitted his resignation to the Council which the Council accepted. The Council appointed Dr. Helen Hart as Editor-in-Chief of PHYTOPATHOLOGY to fill the unexpired term (through 1945) subject to the approval of the Society at the next annual meeting.

W. H. Burkholder, A. J. Riker and W. J. Zaumeyer were appointed editors of PHYTOPATHOLOGY, and A. A. Dunlap, Helen Hart, W. G. Hoyman and H. N. Racicot were appointed associate editors of PHYTOPATHOLOGY—all for a three year term.

R. M. Caldwell was appointed Business Manager of PHYTOPATHOLOGY for a three year term, and S. L. Hopperstead, Advertising Manager, for a one year term.

Reports of Officers, Representatives, and Committees. The Council recommended acceptance of the reports, which were approved by the Society. Reports of officers, representatives, and standing committees are published on the previous pages. According to action of the Society at the Philadelphia meeting only reports of the standing committees are to be published in the annual report.

Tropical Research Foundation. The Society confirmed the Council's recommendation to accept the final report of our representative, Dr. R. D. Rands, with appreciation of the active work carried on by our representative and that the representative be excused from further service. The announcement of the "Dissolution of the Tropical Plant Research Foundation" was published in an earlier issue of PHYTOPATHOLOGY.

Special Committee—Coordination of Cereal and Vegetable Seed Treatment Research. The Society confirmed the Council's recommendation that the names of members of the sub-committees of the Special Committee on "Coordination of cereal and vegetable seed treatment research" not be published in PHYTOPATHOLOGY. The chairman of this committee was authorized to add members to this committee when necessary during the year. The Council expressed its appreciation in the name of the Society for the faithful and important work that this committee is doing.

Publication of Monographs. The Council recommended and the Society approved that the Special Committee on "Publication of Monographs" be discharged.

Recommended Methods. A report on "recommended methods" by the committee on "Standardization of fungicidal tests" was accepted by the Society, and the Society confirmed the recommendation of the Council to refer this to the Editor-in-Chief of PHYTOPATHOLOGY for consideration relative to publishing in PHYTOPATHOLOGY.

Phytopathology. The Council recommended and the Society approved the appointment of a Special Committee to include the Business Manager of PHYTOPATHOLOGY and the Editor-in-Chief as ex officio members "to make a study of the problems affecting the publication—PHYTOPATHOLOGY."

Membership List. It was moved by the Society that a membership list be published in 1944—the matter of time and how to be left to the discretion of the officers of the Society. President J. J. Christensen appointed a Temporary Committee which has reported on the matter of publishing a membership list, and the report has been accepted by the officers of the Society.

1944 Society Meeting. The Society approved the recommendations of the Council that no official summer meeting be held, but that an annual meeting be held in 1944—the time and place to be decided by the program committee. Since this action of the Society the Council has voted not to hold the annual meeting with AAAS, September 9–16, 1944, Cleveland, Ohio, but to hold a separate meeting early in December when more field data will be available.

ANDRÉS R. LÓPEZ ELÍAS

February 4, 1909–February 4, 1943

Andrés R. López was graduated from the Polytechnic Institute at San Germán, Puerto Rico, in 1928. During 1928–29, he attended the college of Agriculture and Mechanic Arts, University of Puerto Rico, and then transferred to Louisiana University where he received the degree of Bachelor of Science in Agriculture in 1933 and the degree of Master of Science in 1935, majoring in Plant Pathology. During 1939–40, he pursued further graduate study at Iowa State University.

For a time Mr. López served as Agronomist in the Federal Emergency Relief Administration and later taught vocational agriculture in one of the Second Unit Schools of the Department of Education at San Sebastian, Puerto Rico. From 1936 to the time of his death, except for the school year 1939–40, he served as Assistant Pathologist at the Agricultural Experiment Station of the University of Puerto Rico.

Mr. López was a young man of great promise in his chosen field of science and a true friend among his colleagues.

HOWARD EVERETT PARSON

May 13, 1897–November 18, 1943

Howard Everett Parson was graduated from Michigan State College in 1923 with the degree of Bachelor of Science and received the degree of Master of Science from the University of Minnesota in 1928.

During the school year of 1924–25, Mr. Parson was superintendent of and taught agriculture in the Smith-Hughes Consolidated School at Mesick, Michigan. From 1925 to 1928, he was Instructor in Botany and Assistant in Plant Pathology in the University of Minnesota. In 1929, he was appointed Junior Pathologist in the Bureau of Plant Industry, U. S. Department of Agriculture, and worked on diseases of pecans. In 1930, he was advanced to the rank of Assistant Pathologist, and later was placed in charge of investigations of pecan diseases.

Mr. Parson was a man of quiet, retiring disposition, unusually conscientious and dependable in his work. He was unusually congenial in his associations, and made friends wherever he went.

STAUFFER SULPHURS

U L P H U R

T A U F F E

"3-M" Magnetic Micron Measured Sulfur (Dry Wettable)

"Magnetic-Spray" Wettable Sulfur

"Magnetic" Catalytic Sulfur

"Magnetic" Humidust

"Magnetic" Cryolite

"Magnetic" Cryolite Dusts & Sprays

"Magnetic" Carbon Bisulphide

"Magnetic" Carbon Bisulphide Emulsion

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STUDIES ON LILY VIRUS DISEASES: THE NECROTIC-FLECK COMPLEX IN LILIUM LONGIFLORUM

PHILIP BRIERLEY[1] AND FLOYD F. SMITH[2]

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The necrotic fleck disease in Easter lilies (*Lilium longiflorum* Thunb.) is capably described in an early account by Stewart (19) who distinguished it from Botrytis blight, and found the necrotic streaks were not due to insect or fungus attack. Necrotic fleck symptoms are included in Woods' account (22) of "The Bermuda Lily Disease," and illustrated in a second paper by that author (23). Neither of these early workers suggested the virus etiology of the disease.

Ogilvie and Guterman (17) described a mosaic "disease or group of diseases" in Bermuda Easter lilies, distinct from the rosette disease (or yellow flat) (5, 16). They distinguished 3 types of symptoms: A. Marked downward curling of leaves accompanied by short chlorotic streaks becoming rusty necrotic, and flower distortion; B. Similar linear necrotic spotting, with some twisting but no characteristic curling of leaves, and no marked distortion of flowers; C. The leaves mottled, notably stiff and variously distorted, and the flowers aborted or deformed. Ogilvie and Guterman (17) recognized these diseases as of virus origin, and observed evidence of natural spread in Bermuda, but were unsuccessful in attempts to transmit them. Symptom types A and B of these investigators correspond well with the severe and the mild forms, respectively, now recognized in necrotic fleck, but their type C, possibly due to a mottle virus (4), cannot be definitely classed from their description and illustrations.

Guterman first transmitted "the virus of lily mosaic" by inarching and by needle-prick (8), and by *Aphis gossypii* Glover (9, 10). He recognized 3 types of mottling in lilies: (1) linear, light-green areas, drying to more or less linear necrotic spots; (2) elongate chlorotic streaking without necrosis; and (3) chlorotic mottling in rounded or irregular areas without necrosis. The disease we now call necrotic fleck was shown as an example of the first type, and was considered the typical response of Easter lily, while the characteristic symptoms of over 40 other species were assigned to one or another of the above 3 types. Extensive cross-inoculations between species by stem puncture, and a number of successful transfers by *A. gossypii*, seemed to yield uniform symptoms of some one of these 3 types in each species inoculated, regardless of the species serving as source of virus. Guterman (9), therefore, concluded that "No evidence of any sort was obtained during these experiments which would indicate that more than one

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virus is concerned." He did observe, however, that "mosaic affected plants of *Lilium longiflorum* may not necessarily exhibit necrosis of the chlorotic areas on the leaves." Inoculations from various species showing symptom types 2 and 3, as well as from Easter lilies showing type 1, induced type 1 symptoms (*i.e.*, necrotic fleck) in certain of the Easter lilies inoculated. The fact that Guterman used symptomless commercial stock, rather than seedlings, as test material apparently prevented his recognizing the separate significance of mottling and necrotic flecking in Easter lily.

In 1937 Price (18) reported the isolation from commercial Giganteum Easter lilies of a cucumber-mosaic virus, which, in common with other strains of this virus, was capable of inducing necrotic fleck symptoms in certain Easter lilies that "were either grown from seed or were from selected cage-grown stock." Although the necrotic symptom appeared in a low percentage of the Easter lilies he inoculated with cucumber-mosaic virus, Price accepted Guterman's (10) interpretation of lily mosaic as being of single virus etiology and assigned this lily-mosaic virus to the cucumber-mosaic virus group.

McWhorter was the first to show tulips are susceptible to viruses from lilies, reporting tulip breaking induced by juice of mosaic *Lilium speciosum* in 1932 (12), and in 1937 (13), by juice of symptomless *L. candidum* L., *L. longiflorum*, and *L. tigrinum* Ker-Gawl. His latent virus of lily at first (13) considered identical with Tulip Virus 1 (14) was later (15) distinguished from this by cytological methods.

One of us (1), impressed with the distinctness of mottle and necrotic fleck symptoms in the Creole Easter lily, repeated Price's (18) and McWhorter's (13) experiments, finding a tulip-breaking virus in both mottled and flecked Easter lilies, but cucumber-mosaic virus in the necrotic fleck type only. Subsequent work (3) showed that cucumber-mosaic virus alone does not induce necrotic fleck in previously virus-free Easter lily seedlings, but does produce this symptom when introduced into certain symptomless commercial Easter lily stocks. Tulip breaking induced in tulips by cucumber-mosaic virus was also shown to be recognizably different from the type produced by the true tulip-breaking viruses and their lily-mottle allies. Brierley (2) then showed that seedlings of *Lilium formosanum* Stapf are satisfactory index plants for the tulip viruses and, using these and tobacco as test subjects, found both cucumber-mosaic virus and tulip-type viruses widely distributed in lilies, the latter being more common than the former.

Further studies on the lily-mottle viruses or tulip-breaking types will be presented separately (4). The present paper presents evidence that the necrotic fleck disease (NF) is a complex of cucumber-mosaic virus (CV) and a symptomless virus (LSV) not previously reported, that *Aphis gossypii* is a specific vector for LSV, and therefore for the complex CV + LSV, and that mottle viruses (LMV) consistently associated with necrotic fleck in commercial Easter lilies are not an essential part of the fleck complex. Some information is included on the distribution, host range, and economic impor-

tance of LSV, termed lily-symptomless virus and described as *Adelonosus lilii*, nov. gen. et sp.

Preliminary evidence is presented that LSV and lily-rosette virus (5, 16) are of the "persistent" group (20), having latent periods of several days in their common vector *Aphis gossypii;* but that lily-symptomless virus (LSV) does not protect Easter lilies against infection by lily-rosette virus (LRV).

SYMPTOMS OF NECROTIC FLECK

As previously mentioned, Stewart (19) and Guterman (9, 10) have described this disease. The term "fleck" was applied by one of us (1) in order to provide a distinctive name for the complex. Characteristic flecks are variable in size, usually elongated parallel to the veins, chlorotic when first recognized but becoming gray to brown necrotic (Fig. 1). When fully developed the dead areas are depressed but the surface remains intact. The name necrotic fleck (NF) is reserved for this complex in *Lilium longiflorum* in our usage. Other lily species may develop necrotic spotting, as noted by Guterman (9, 10), but only the Easter lily has thus far been shown to develop these symptoms from infection by the virus complex here discussed.

Dwarfing, curling (Fig. 2), and flower deformities (Fig. 3) also accompany the typical NF. The stature is often less than half that of symptomless plants of the same variety. Leaves may be curled downward as well as flecked, but this curling is less regular and commonly less extreme than that characterizing lily rosette disease (16). Flowers are subnormal in size, fail to open fully, and are variously distorted by thin streaks which become brown necrotic by the time the flower is fully developed (Fig. 3). Such typical NF individuals, comparable to Type A of Ogilvie and Guterman (17), are unsalable. After flowering, and sometimes before, NF plants begin to lose leaves from below upward. Such leaves first turn pale-yellow with irregular green blotches, then wither away. Affected plants mature earlier than symptomless individuals of the same variety, and yield small bulbs. Such bulbs are subject to rot, and the survival of the NF type is low. The NF symptoms always persist, in those individuals that do survive, during the next season of growth.

A milder sub-type, scored as "sparse fleck" (SpF) in our records, is comparable to type B of Ogilvie and Guterman (17). In this type the necrotic flecks are fewer and usually larger than in the severe type, there is less marked dwarfing, the leaves are twisted irregularly instead of curled downward, and the flowers are seldom distorted. SpF plants are often considered salable, particularly in years of deficient supply. Experimentally produced flecking is often of this SpF type (Fig. 4), and the illustrations Price (18) published may be so classed. SpF Easter lilies grown in screened and fumigated greenhouses for several successive seasons are fairly stable in symptom expression, but some NF may develop in these under such conditions.

A still milder type, which we term "Very Sparse Fleck" (VSpF), may be detected in certain commercial Easter lilies under glass, but is doubtless

overlooked in the field where various injuries may be confusing. No dwarfing, curling, or flower distortion accompanies the very sparsely distributed necrotic flecks; affected plants are, therefore, salable. The VSpF type selected from commercial Creole lilies forced in the season of 1939–40 and

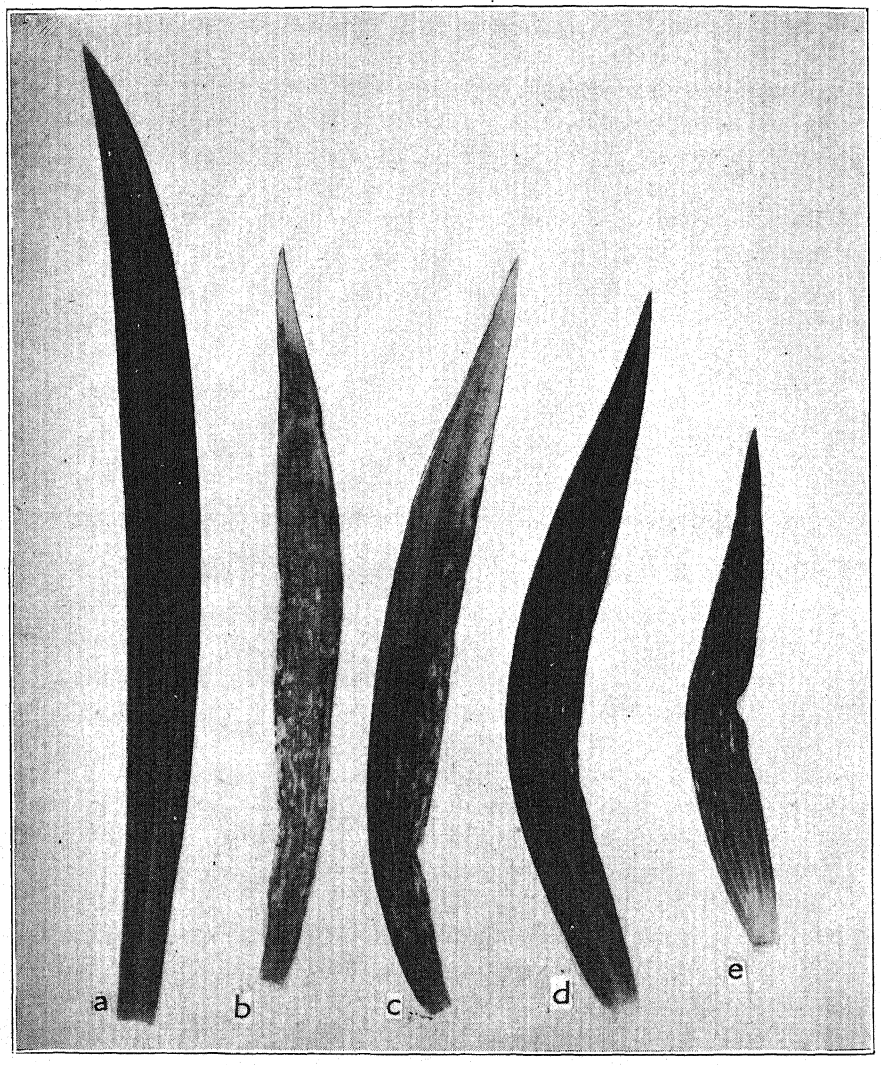


FIG. 1. Leaves of an Easter lily seedling experimentally infected with necrotic fleck by *Aphis gossypii:* (a) normal lower leaf, (b) first of the group of leaves showing advanced necrosis and yellowing, (c, d, e) younger leaves in less advanced stages of fleck symptoms. Photographed by Pratt.

grown again under glass in 1940–41 produced 1 symptomless individual, 1 VSpF, and 15 severe SpF plants, of which 13 were judged unsalable because of flower distortion. Ten VSpF plants selected from forced Creole lilies in 1941–42 and grown again under glass in 1942–43 produced 3 symptomless, 3 VSpF, and 4 SpF plants, of which 3 were considered unsalable.

Attention is called to the two sub-types of necrotic fleck, SpF and VSpF, as confusing factors in the estimation of fleck in forced Easter lilies, and as obstacles to effective roguing in the field. Although examples of the 3 types of fleck described here can be found in nearly any affected commercial stock



FIG. 2. Necrotic fleck in Easter lily: (a, b) experimentally infected with cucumber-mosaic virus and lily-mottle virus in February, 1940, no fleck resulting. Lily-symptomless virus added to (a) by *Aphis gossypii* in February, 1941. Photographed in April, 1942; in 6-inch pots.

of Easter lilies, the sub-types are not separable one from another by sharp dividing lines, and all transition stages from the mildest VSpF to the most severe NF types occur. These types cannot be distinguished at present by their virus content, all 3 yielding CV and LMV on indexing, and all

evidently carrying LSV which is now held essential to the expression of NF symptoms. Attempts to demonstrate another factor for dwarfing in the severe NF type have not been successful, and the virulence of the complex has not been found consistently correlated with the virulence of the CV component.

The milder necrotic fleck types (SpF, VSpF) are commonly ignored by producers of Creole Easter lilies, who recognize the severe or typical NF type



FIG. 3. Symptoms of necrotic fleck in Creole Easter lily flower. The flower is small (4.5 inches long and 4.5 inches across), the perianth distorted and streaked with brown. Natural infection. Photographed by Allard.

only. It is true that this type only is consistent in producing cull types when forced, but the evidence presented above indicates that all 3 types represent essentially the same disease complex, and that shifts from the milder to the more severe types may be expected without additional contamination from outside sources.

MECHANICAL TRANSMISSION

Sap inoculation experiments of 5 categories are summarized in table 1. When cucumber-mosaic virus (CV) was inoculated into supposedly healthy Easter lily seedlings, only 1 plant of 512 developed necrotic fleck symptoms.

This one positive result may be discounted as contamination, inasmuch as the California-grown seedlings inoculated here showed 3 NF individuals in 750 plants previous to this test. It is reasonable to suppose that a few addi-

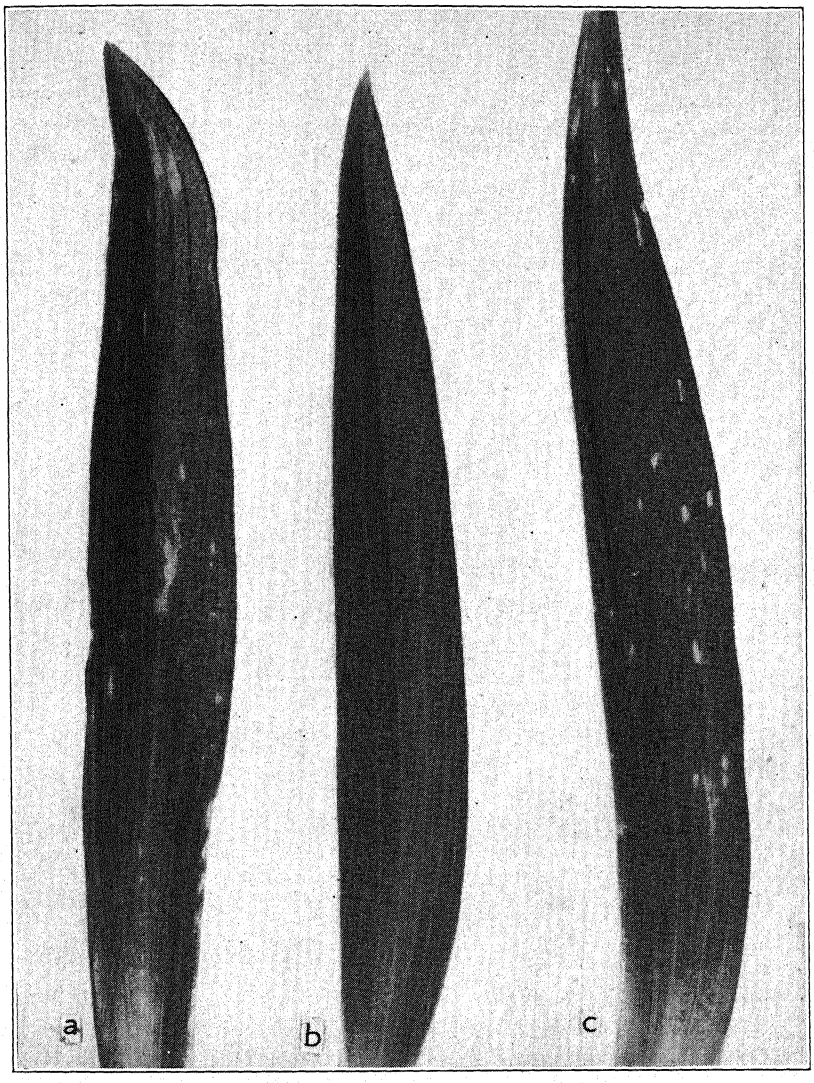


FIG. 4. Sparse fleck sub-type of necrotic fleck experimentally produced in Easter lily seedlings: (a, b, c) mechanically inoculated with cucumber-mosaic virus and lily-mottle virus in December, 1940. Lily-symptomless virus added by *Aphis gossypii* to (a) in March, 1941, and to (c) in January, 1942. Photographed in May, 1942 (natural size).

tional seedlings of this lot were contaminated with LSV which with CV produces NF. It is also reasonable to conclude that Price (18) was able to produce NF symptoms on inoculation with CV alone because some of the symptomless Easter lilies he inoculated carried LSV. The available evidence,

therefore, justifies the conclusion that CV alone cannot produce NF in healthy Easter lilies.

When CV was inoculated into symptomless commercial Easter lilies (LMV + LSV) a high proportion of necrotic fleck (97 in 132) resulted. Characteristic symptoms were thus produced in symptomless Creole Easter lilies (the Norwood strain and a Florida strain), and in symptomless Croft lilies, as well as in plants of Creole and Giganteum varieties that showed mottling. Inasmuch as all these commercial stocks, whether mottled or not, can be shown by indexing to carry LMV, the evidence thus far presented would justify the hypothesis that LMV and CV in combination produce NF. In some trials some strains of CV failed to induce NF under conditions appar-



FIG. 5. Necrotic fleck mechanically transmitted to Easter lily seedling (left) compared with control seedling (right). Photographed in 6-inch pots 55 days after inoculation.

ently favorable for infection, and cucumber appeared to be a poor source of CV for inoculation into lilies, but 100 per cent infection has been approached or attained in many such inoculations with more virulent strains of CV from tobacco.

Several combination inoculations with CV and LMV into healthy Easter lily seedlings failed to induce necrotic fleck. In separate trials preparations containing CV and LMV were mixed and inoculated simultaneously, or the two were introduced separately into different shoots of double-nosed Easter lily seedlings, or the one was introduced alone and then followed after 20 days by the other. No fleck resulted from any of these tests, although the inoculated seedlings were held for a year or more and subinoculations revealed both CV and LMV present. It was, therefore, concluded that the com-

bination of CV and LMV does not produce NF. It was further postulated that a third constituent (LSV), absent from suitably protected Easter lily seedlings but present in commercial Easter lilies, must be essential for the expression of necrotic fleck symptoms.

TABLE 1.—*Mechanical inoculation of Easter lily with necrotic fleck (NF), or with the cucumber-mosaic component (CV), or with CV and lily-mottle virus (LMV) together*

Source plant	Virus or complex	Plants inoculated	Total tests	No. of tests positive	Plants flecked[a]	Minimum incubation period
		A. CV on healthy Easter lily seedlings				
						Days
Tobacco	CV	Easter lily sdlgs.	78	1	1/460	38
Easter lily	"	"	3	0	0/22	.....
Cucumber	"	"	2	0	0/30	.....
		B. CV on commercial Easter lilies (LMV + LSV)				
Tobacco	CV	Creole (symptomless)	22	19	79/98	13
Easter lily	"	" "	1	1	5/5	20
Easter lily	"	" (mottled)	1	1	2/5	25
Easter lily	"	Croft (symptomless)	1	1	2/5	20
Cucumber	"	" "	1	0	0/5	.....
Tobacco	"	" "	2	1	4/9	33
Tobacco	"	Giganteum (mild mottle)	1	1	5/5	33
		C. CV + LMV on healthy Easter lily seedlings				
Easter lily	CV + LMV	Easter lily sdlgs.	15	0	0/175	.....
Cucumber + E. lily	"	"	1	0	0/3	.....
		D. NF on healthy Easter lily seedlings				
Commercial Easter lilies	NF	Easter lily sdlgs.	17	3	6/144	31
Easter lily sdlgs.	"	"	7	4	5/160	44
		E. NF on commercial Easter lilies (LMV + LSV)				
E. lily sdlgs.	NF	Creole (symptomless)	3	3	23/25	13
"	"	" (mottled)	1	1	9/10	47
"	"	Croft (symptomless)	1	1	7/10	29

[a] Expressed as number flecked over number exposed (mottling not recorded here).

Necrotic fleck was sap-transmitted to healthy Easter lily seedlings in low proportion, 6 in 144 from naturally-infected source plants, and in about the same proportion, 5 in 160, from flecked material experimentally produced by previous mechanical transfer or by aphid transfer (Fig. 5). No infection resulted in trials in which the inoculum was prepared with phosphate buffers at pH 6, 7, or 8, or in saline solution, the successful transfers being pre-

pared with tap water. The low percentage of transfer (3.6 per cent) here recorded is considered authentic. Contamination of the test seedlings with the symptomless component (LSV) of fleck, as discussed above (Sect. A, Table 1), cannot explain the 11 positive flecks recorded here, for seedlings used in most of the positive tests were rigidly protected, some having been grown in screened and fumigated greenhouses throughout. Occasional seed-carriage of LSV may also be dismissed as an explanation of occasional transfer, as such seed-borne virus might be expected to occur with as great frequency in the 512 seedlings in Sect. A, of Table 1, or in the 178 in Sect. C, as in the 304 seedlings of Sect. D. It is therefore concluded that the NF complex is mechanically transmissible as such, but with difficulty. This difficulty of transfer is in itself further evidence for the existence of an additional essential component (LSV), as both CV and LMV are readily transferred mechanically.

Necrotic fleck is readily transferred to commercial Easter lilies of the Creole and Croft varieties, and presumably to other varieties not tested. This finding was to be expected as only CV is necessary to induce fleck in such stocks, as is shown above (Sect. B, Table 1). Comparison of the data summarized in Sections D and E of table 1 reveals a sharp contrast between the difficulty of transmitting NF to seedlings and the ease of transmitting it to commercial stocks carrying LMV + LSV. This contrast is held to be due to the nearly universal presence of LSV in commercial Easter lily stocks. Guterman's (9) ready transfer of a disease now interpreted as NF from Easter lily to Easter lily was evidently comparable to our experience (Sect. E, Table 1), although he used the Harrisii and what was probably Erabu (*Lilium longiflorum formosum*) varieties, which were not included in our tests.

TRANSMISSION BY APHIDS

Studies on vector relations of necrotic fleck and other virus diseases were undertaken in 1939. Some of the data on transmission of NF and its components CV and LSV are summarized in table 2. Transmission of the NF complex was determined by symptom expression in Easter lily, and transmission to other species was determined by return transfer to Easter lily by *Aphis gossypii*. Transmission of LMV was detected by characteristic symptom expression in Easter lily, *Lilium formosanum,* or tulip, or by mechanical subinoculation to one of the two plants last named. Transmission of CV was proved by subinoculation to tobacco.

In these experiments *Aphis gossypii* was confirmed as a vector of the NF complex (Fig. 6). Six other species of aphids failed to transmit this complex as such, but *Macrosiphum solanifolii* Ashm. and *Myzus persicae* Sulz. were found to transmit both CV and LMV. These findings are in agreement with those of sap transfer tests (table 1) in showing that CV and LMV together fail to produce NF, and that a further constituent (LSV) must be essential to expression of NF symptoms. They further indicate that *A. gossypii* is a specific vector of the LSV constituent, for this species is certainly not specific for CV.

Possible transmission of the NF complex to tulip, *Colchicum,* and *Gloriosa* is indicated in table 2. No distinctive symptoms were detected in tulip be-

TABLE 2.—*Transmission of necrotic fleck (NF), or of cucumber-mosaic virus (CV) or mottle virus (LMV) from flecked Easter lily to various plants by aphids*

Plants exposed	Tests		Plants affected[a]	Minimum NF incubation period	Virus transmitted		
	Total	Positive for NF			NF complex	CV	LMV
By *Aphis fabae* Scop.							
	No.	*No.*		*Days*			
Easter lily sdlgs.	1	0	0/6	.....	.....	.....	.....
Tulip (Clara Butt)	1	0	0/4	.....	.....	.....	.....
By *Aphis gossypii* Glover							
Easter lily sdlgs.	33	22	112/250	14	+	+	+
L. formosanum Stapf	3	0	0/16	.....	.....	+	+
L. tigrinum Ker-Gawl	2	0	0/14	.....	.....	+	.....
Calochortus spp.	2	0	0/10	.....	.....	+	+
Colchicum autumnale L.	3	1	2/16	53	.....(?)	+	.....
Gloriosa rothschildiana O'Brien	4	3	14/25	10	.....(?)	+	.....
Fritillaria pudica Spreng.	1	0	0/5	.....	.....	+	.....
Crimson clover	1	0	0/5	.....	.....	+	.....
Tulip (Clara Butt)	7	5	28/78	21	.....(?)	+	+
By *Macrosiphum lilii* Monell							
Easter lily sdlgs.	7	0	0/45	.....	.....	.....	.....
L. formosanum	1	0	0/5	.....	.....	.....	.....
Tulip (Clara Butt)	1	0	0/8	.....	.....	.....	.....
By *Macrosiphum solanifolii* Ashm.							
Easter lily sdlgs.	8	0	0/85	.....	.....	+	+
Tulip (Clara Butt)	2	0	0/14	.....	.....	.....	.....
By *Myzus circumflexus* Buckt.							
Easter lily sdlgs.	3	0	0/26	.....	.....	.....	.....
L. formosanum	1	0	0/5	.....	.....	.....	.....
Tulip (Clara Butt)	1	0	0/17	.....	.....	.....	.....
By *Myzus convolvuli* Kalt.							
Easter lily sdlgs.	2	0	0/8	.....	.....	.....	.....
By *Myzus persicae* Sulz.							
Easter lily sdlgs.	12	0	0/116	.....	.....	+	+
Tulip (Clara Butt)	8	0	0/95	.....	.....	+	+

[a] Number of plants expressing NF over number exposed.

yond those known to be characteristic of CV infection, but two return transfers from the exposed tulips to Easter lily seedlings by *Aphis gossypii* pro-

duced typical NF. In *Colchicum autumnale* L. necrotic streaks appeared at the base of leaves in the region that was in active growth immediately after exposure (Fig. 7). CV was recovered mechanically, but a return transfer of *A. gossypii* to Easter lily seedlings yielded no NF. Attempts to induce these symptoms in *Colchicum* with CV alone have thus far proved unsuccess-

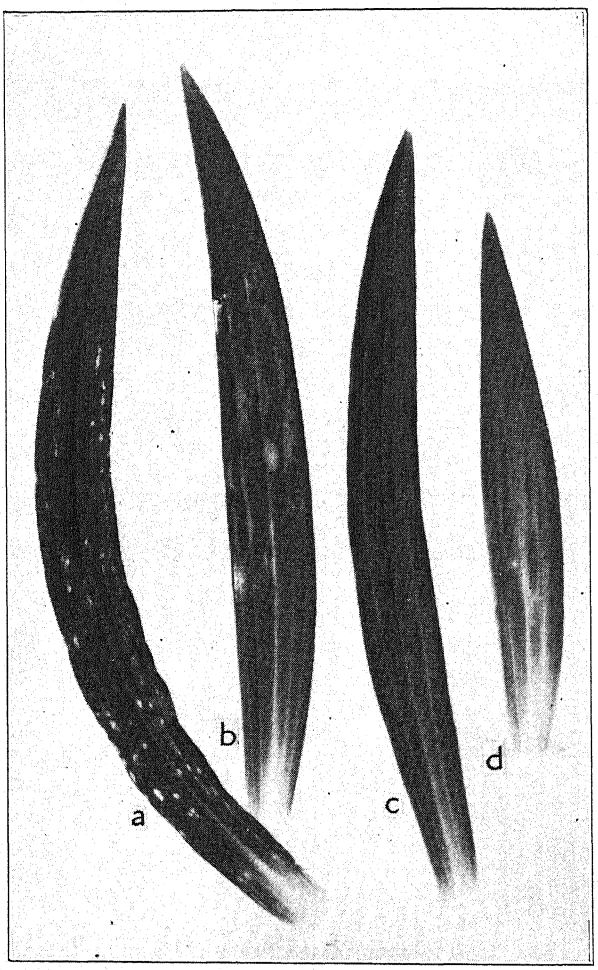


FIG. 6. Symptoms induced in Easter lily seedlings by aphid transfer from necrotic-fleck-infected Easter lilies: necrotic fleck transmitted by *Aphis gossypii* (a); lily-mottle virus transmitted by *Myzus persicae* (b); by *Macrosiphum solanifolii* (c), and by *Aphis gossypii* (d). Photographed by Pratt.

ful. *Gloriosa rothschildiana* developed striking yellow ring patterns in leaves after transfer of this vector from NF Easter lilies (Fig. 8). CV was recovered from such plants, and somewhat similar but less prominent rings were induced in *Gloriosa* seedlings by inoculation with CV only. One of 5 return transfers to Easter lily seedlings by *A. gossypii* produced NF. Unfortunately the apparently successful return transfers from both *Gloriosa*

and tulip were made to Easter lily seedlings that had been inadequately isolated in Southern California and carried some natural infection with NF and presumably also some additional infection with LSV. It is therefore uncertain whether necrotic fleck was returned to Easter lily from tulip and *Gloriosa* or whether CV was returned to seedlings already contaminated with LSV.

Cucumber-mosaic virus was recovered also from symptomless plants of *Lilium tigrinum, Calochortus* sp., and *Fritillaria pudica* exposed to fleck

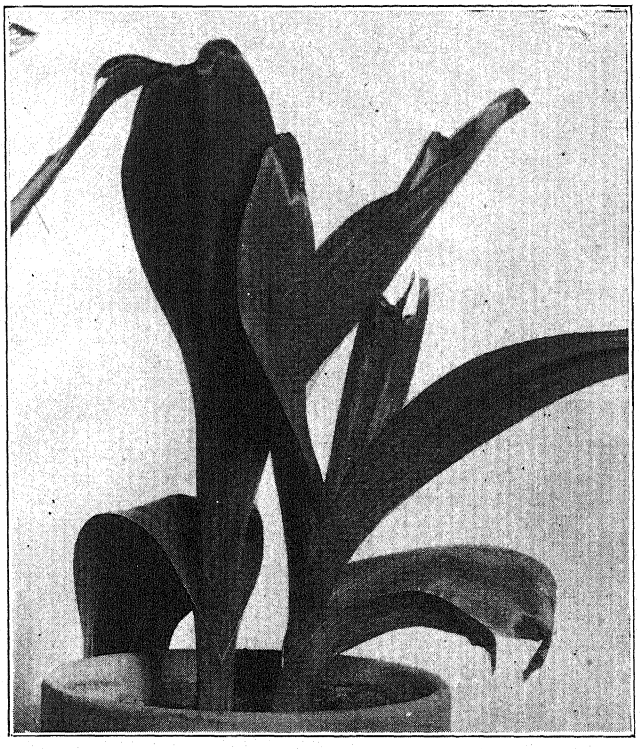


FIG. 7. Symptoms induced in *Colchicum autumnale* by transfer of *Aphis gossypii* from necrotic-flecked Easter lily. Cucumber-mosaic virus was recovered. Photographed (in 6-inch pot) by Guernsey.

by *Aphis gossypii.* This virus was previously reported (2) as occurring naturally in *L. tigrinum. Calochortus* sp., *Colchicum autumnale, Gloriosa rothschildiana,* and *F. pudica,* are believed to be previously unreported as hosts of CV. The other plants listed as affected in table 2 have been previously reported susceptible.

The plants reported susceptible to lily-mottle virus in table 2 were previously known to be subject to this virus (4). In addition, *Lilium tigrinum* and *Fritillaria pudica* are susceptible; failure to develop symptoms in the tests recorded here is further evidence that *Aphis gossypii* is a poor vector of LMV. *Colchicum, Gloriosa,* and crimson clover have not proved to be susceptible to LMV.

In experiments conducted over a period of 4 seasons, *Aphis gossypii* transmitted NF, or its component LSV, to Easter lily seedlings somewhat irregularly. The irregularities cannot be fully explained, but a number of factors seems to be operative. First, all trials during the period of higher greenhouse temperatures from late April to early winter failed, even when succulent lateral shoots were exposed to the aphids. Secondly, *A. gossypii* seems unable to establish itself and thrive on the young leaves of Easter

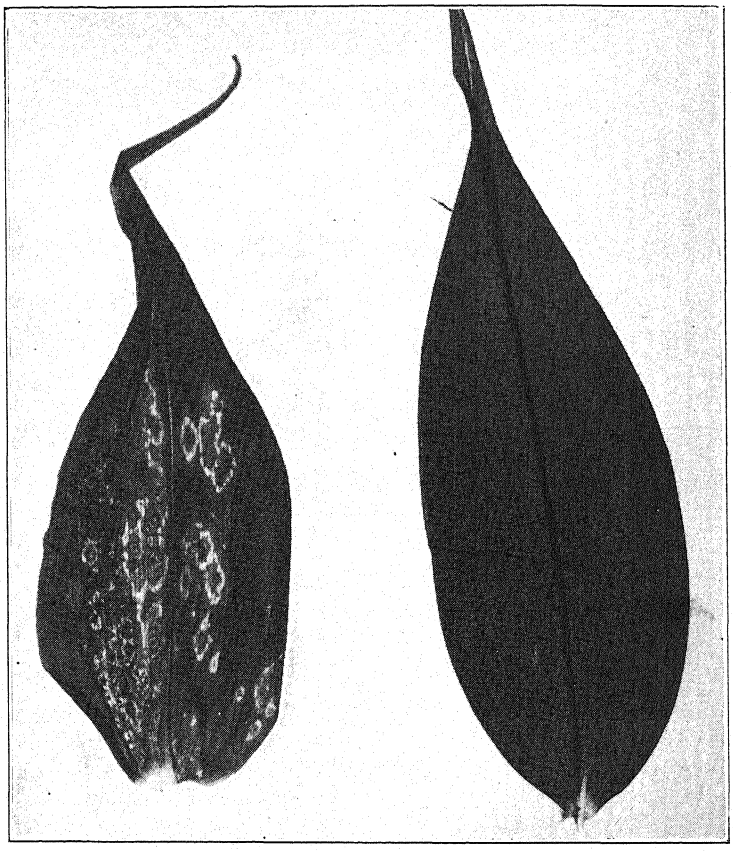


FIG. 8. Symptoms induced in *Gloriosa rothschildiana* by transfer of *Aphis gossypii* from necrotic-flecked Easter lily (left) in comparison with control (right). Cucumber-mosaic virus was recovered. Photographed by Skeris.

lilies under the cooler conditions of fall and early winter, but more readily accepts leaves and flower buds in later stages of growth. Furthermore, even in winter and early spring, when transmission has been most consistent, the percentage of transfer has varied, suggesting that the concentration of one or both of the constituent viruses, CV and LSV, may vary in the Easter lily source plants.

Recent experiments show that LSV is of the "persistent" group as defined by Watson and Roberts (20), having a latent period of several days

in the vector *Aphis gossypii,* and persisting for at least 3 days in the vector on non-susceptible plants. Cucumber-mosaic virus, on the other hand, has been shown by Watson and Roberts (20) to be a "non-persistent" virus, surviving for a brief time in aphid vectors, and lacking a latent period in these. Inasmuch as the requirements for efficient transmission of viruses of these two groups are quite different, the transmission of a complex consisting of one of each group (CV + LSV) might be expected to be somewhat erratic. It was shown in one test that *A. gossypii* may transmit LSV

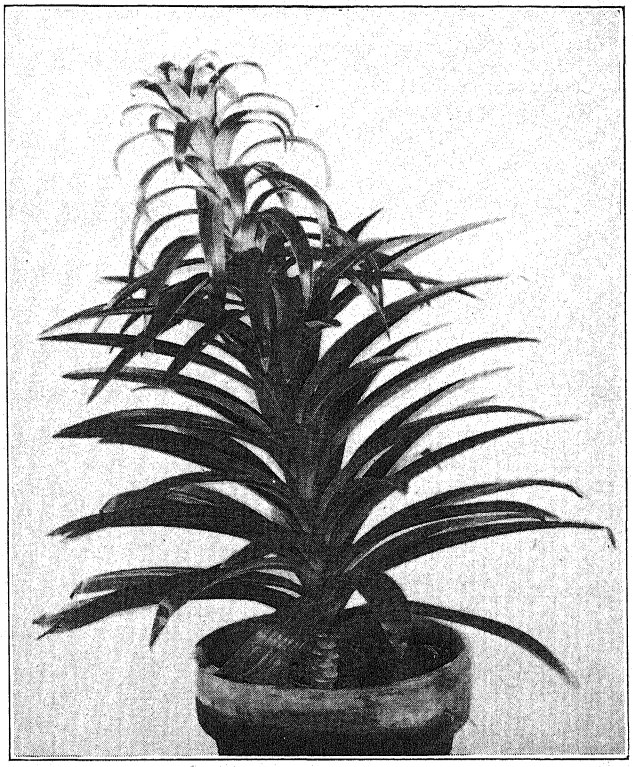


FIG. 9. Lily-rosette virus superimposed on Norwood Creole Easter lily naturally infected with lily-symptomless virus. *Aphis gossypii* transfer. Photographed (in 5-inch pot) 36 days after exposure.

without CV from NF source plants that carry both. One of 5 plants exposed developed NF symptoms, but the subsequent addition of CV to the remaining 4 plants induced one more to express necrotic fleck.

Lily-rosette virus (5, 16), received for diagnosis in Easter lily from Florida, was compared with LSV in some experiments. The data in table 3 indicate a latent period of 3 to 7 days in the aphid vector for lily-rosette virus (LRV), and one of 4 to 6 days for lily-symptomless virus. Similarly the data in table 4 show persistence of LSV in the vector *Aphis gosspyii* for at least 3 days, and of LRV for at least 9 days. The two viruses LRV and LSV are similar in most known characteristics, except that LRV produces

TABLE 3.—*Evidence of a "latent" period within the vector Aphis gossypii as essential for transmission of lily-rosette virus, lily-symptomless virus, and the necrotic-fleck complex from Easter lily to Easter lily*

Latent period (days)[a]	Virus or complex transmitted								
	Lily-rosette virus			Lily-symptomless virus			Necrotic-fleck complex		
	Total tests	Positive tests	Plants affected[b]	Total tests	Positive tests	Plants affected[b]	Total tests	Positive tests	Plants affected[b]
2	...	...	.....	1	0	0/10	1	0	0/10
3	1	0	0/10	...	...	.....	3	0	0/21
4	...	...	.....	...	...	.....	3	0	0/13
6	...	...	.....	2	0	0/16	...	...	.....
7	...	...	.....	...	...	.....	1	1	2/5
8	2	1	1/12	1	0	0/14	2	2	9/10
9	...	...	.....	...	...	.....	2	1	3/10
10	1	1	10/10	...	...	.....	1	1	3/10
Over 10	6	6	35/38	13	10	47/125	25	22	120/217

[a] Total elapsed time of feeding on both source plant and test seedling.

[b] Number of plants affected over number exposed. The transmission of lily-symptomless virus was determined by mechanical addition of cucumber-mosaic virus to the test seedlings either before or after these were exposed to feeding of *Aphis gossypii* from Norwood Crole lilies.

well-marked symptoms in the Easter lily while LSV produces none. Easter lilies carrying the symptomless virus, however, are not protected against infection by lily-rosette virus (Fig. 9).

In trials with *Aphis gossypii* conducted under apparently favorable conditions 93.8 per cent of 48 plants were infected with LRV, 54.7 per cent of 247 plants with the NF complex, and 37.6 per cent of 125 plants with LSV. The lily-rosette virus, apparently a single entity, is carried with much higher efficiency than is the NF complex. The low percentage transfer of LSV alone presumably indicates that the test for its presence, namely mechanical addition of CV, is not always highly efficient.

It was noted in some of our earliest trials that the mottle virus (LMV) could not be recovered from many of the Easter lily seedlings typically flecked as a result of *Aphis gossypii* transmission. Again in a recent series

TABLE 4.—*Evidence of the retention of lily-rosette virus and of lily-symptomless virus by Aphis gossypii, when first fed on Easter lily source plants, then on non-susceptible plants, and finally on Easter lily test seedlings*

Interval on non-susceptible plants		Retention of virus as shown by final feeding on Easter lily seedings[a]
Species	Days	
Lily-rosette virus		
Chrysanthemum	3	5/5
Tulip	7	3/7
Tulip and Chrysanthemum	7 + 2	5/5
Lily-symptomless virus		
Chrysanthemum	3	4/10[b]

[a] Plants affected over number exposed.
[b] Determined by expression of necrotic-fleck symptoms after addition of cucumber-mosaic virus.

of subinoculations LMV was detected in only 2 of 10 typical examples of necrotic fleck induced by this vector. Similarly, lily-rosette virus as received from Florida in commercial Easter lilies was found to be accompanied by LMV, but transfers to seedlings by *A. gossypii* were usually free from LMV. Evidently LMV is not essential for expression of necrotic fleck symptoms (Fig. 10), or for expression of rosette symptoms (Fig. 11), although it has been found generally present in commercial Easter lilies affected with necrotic fleck or rosette. Evidence presented elsewhere (4) shows that *A. gossypii* is a relatively inefficient vector of LMV. This vector, therefore, offers a means of separating LSV or LRV from the LMV that commonly accompanies them in commercial Easter lilies. Preliminary evidence recently gained indicates that these separations are highly efficient when a part of the latent period in *A. gossypii* is caused to elapse while the vector feeds on a plant such as chrysanthemum that is non-susceptible to LMV.

ANALYSIS AND SYNTHESIS OF NECROTIC FLECK IN EASTER LILIES

Of the three viruses (CV, LSV, LMV) commonly present in commercial samples of necrotic fleck, CV is readily separable by passage through tobacco,

as the other 2 viruses fail to infect this plant. LMV has been separated from the CV and LSV by mechanical inoculation to tulip and thence to lily. When this separation was first reported (1), tulips were not known to be susceptible to CV, and tulip was believed to be a differentiating host. It was

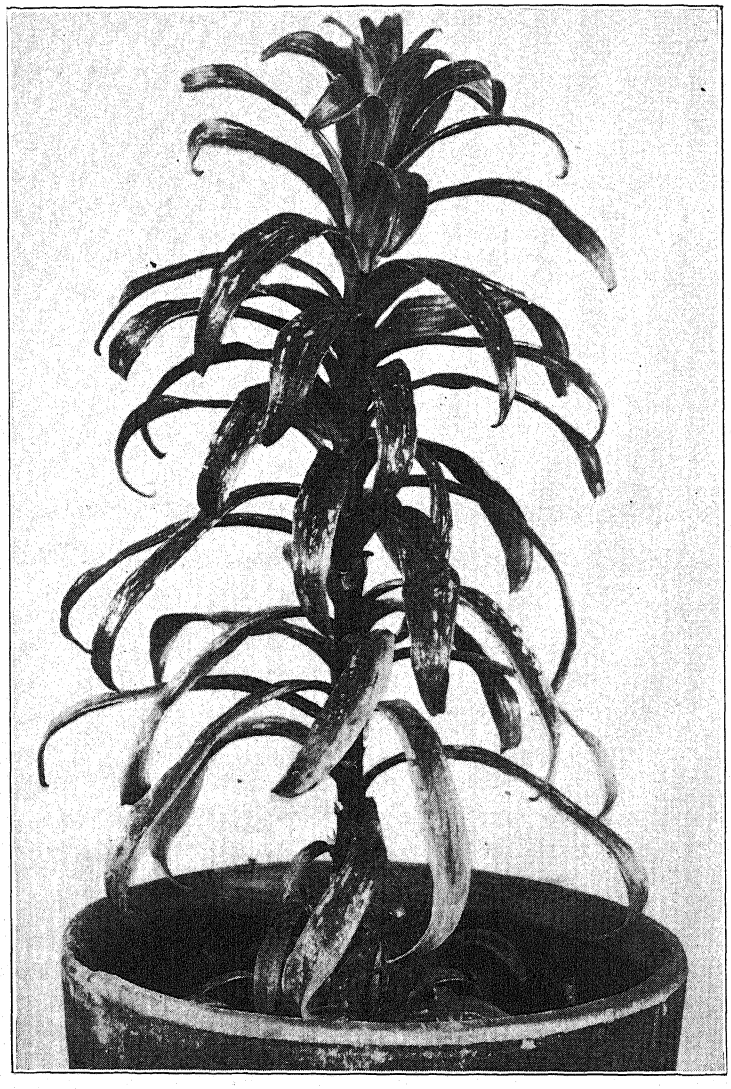


FIG. 10. Symptoms of necrotic fleck in Easter lily seedling induced by *Aphis gossypii*. Subinoculations show lily-mottle virus absent. Photographed (in 5-inch pot) 2 years after exposure.

subsequently found (3) that tulip is susceptible to CV. The fact that this separation succeeded may be attributed to chance failure of CV in the inoculation to or from tulip, or to more rapid movement of LMV in tulip. No better means of separating LMV from CV is known, as all plants known to be

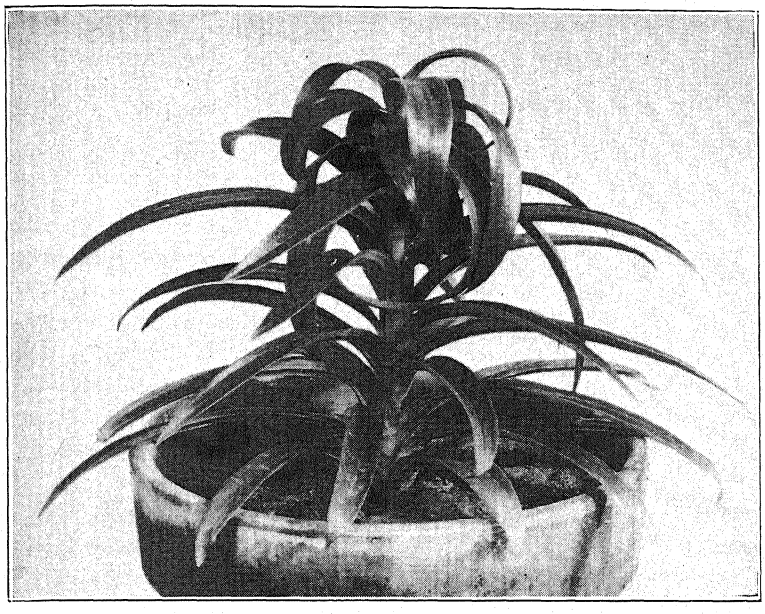


FIG. 11. Symptoms of lily rosette (yellow-flat) induced in Easter lily seedling by *Aphis gossypii*. Subinoculations show lily-mottle virus absent. Photographed (in 5-inch pot) 34 days after exposure.

susceptible to the former are also susceptible to CV, and the properties of these 2 viruses are similar. Separation of LSV from the others is accomplished by *Aphis gossypii* under certain conditions, as discussed above.

In practice the separation of LMV and LSV from CV is not necessary, as these two occur in the absence of CV in certain commercial stocks of Easter lilies that show no fleck symptoms. We have made use of the "Norwood" clonal line of Creole Easter lily, first selected and propagated at Louisiana State University by Dr. Fred Cochran, and further increased and maintained as a known stock at the Plant Industry Station, Beltsville, Md. This usually symptomless clon yields LMV on mechanical transfer and LSV (rarely LMV

TABLE 5.—*Synthesis of necrotic fleck in Easter lily seedlings by sap transfer of cucumber-mosaic virus followed by Aphis gossypii transfer of lily-symptomless virus*

Cucumber-mosaic virus, by sap			Lily-symptomless virus, by *Aphis gossypii*			Necrotic fleck developing in	
Source	Date	Plants inoc.	Source	Date	Plants inoc.	Inoc. plants[a]	Control plants[a]
	(1940)			(1941)			
Easter lily	Feb. 8	24	Norwood Easter lily	Feb. 21	10	8/10	0/14
Tobacco	Dec. 18	25	"	Mar. 12	10	10/10	0/15
				(1942)			
Tobacco	Dec. 18	10	"	Jan. 29	5	4/5	0/5

[a] Expressed as number of plants infected over number treated.

also) on transfer by *Aphis gossypii*. The presence of LSV was first postulated from the fact that this clon yields fleck on addition of CV while seedlings do not (Table 1).

In the experiments summarized in table 5, CV + LMV were first established in Easter lily seedlings as a test of the capacity of these 2 viruses to induce NF. No NF symptoms appeared, as already indicated (Table 1). After 3 to 13 months' incubation, the plants in each test were divided into 2 lots, 1 lot then being exposed to *Aphis gossypii* from Norwood Creole Easter lilies known to carry LSV and LMV. Twenty-two of the 25 plants exposed to infection by both CV and LSV developed necrotic fleck symptoms 33 to 37 days after the second inoculation (Figs. 2, 4), while 34 controls showed no fleck. CV was found present in 16 of 18 control plants indexed in 1943. It is evident from these experiments that a symptomless component LSV essential for necrotic fleck was present in the Norwood Easter lilies, and that this component was carried by *A. gossypii*.

Because of the design of these particular tests, LMV was also present in the fleck synthesized. It should be equally feasible to synthesize necrotic fleck from the 2 essential components (LSV + CV) without LMV. This has been accomplished in a number of trials, but the percentage of necrotic fleck resulting has been low. Low proportions of necrotic fleck in attempted syntheses are considered due to low virulence of CV in some inocula, and also to peculiarities in the feeding habits and virus relations of the vector *Aphis gossypii*, as has been discussed above.

It is considered feasible to produce pure cultures of LSV in Easter lily seedlings by the selective transfer of this virus from Norwood Easter lilies by *Aphis gossypii*. Unfortunately the only available test for the presence of LSV is to add CV, the production of fleck symptoms signifying the presence of LSV. If aphid transfers are made in 1 season and test inoculations in the following season after the bulbs have been divided, it is possible to establish the presence of LSV by inducing NF in 1 division of a test plant while retaining another division as a pure culture of LSV.

In one experiment necrotic fleck was synthesized by two species of aphids, *Myzus persicae* transmitting CV and *Aphis gossypii* transmitting LSV. Nine Easter lily seedlings were first exposed to *M. persicae* from a Creole lily showing NF symptoms (CV + LSV + LMV), with the resulting appearance of mottling (CV + LMV) but no flecking. Later *A. gossypii* was transferred from Norwood Creole (LSV + LMV) to these 9 seedlings. Four of the 9 plants developed necrotic fleck symptoms (CV + LSV). This experiment would suggest the possibility of spread of necrotic fleck by *M. persicae* in commercial fields of Easter lilies (LSV + LMV), in which LSV is general and CV is occasional in necrotic flecked individuals (CV + LSV + LMV). In such lily fields there is every reason to expect spread of CV with the resultant appearance of necrotic fleck, even in the absence of the vector *A. gossypii*, for *M. persicae* and other vectors can transfer CV, the only constituent that is lacking. If such vectors of CV move across lily fields when migrating from

other crops, rapid dissemination of CV comparable to that noted for LMV (4) may be expected.

HOST RANGE OF NECROTIC FLECK

As indicated in table 2, only Easter lily is established as a suscept of both constituent viruses of necrotic fleck (CV and LSV), while the susceptibility of tulip, *Colchicum*, and *Gloriosa* to LSV is left in doubt. Early in the present study a number of different plant species, chiefly Monocots, were exposed to necrotic fleck inoculation by *Aphis gossypii*. It was believed at the time that such tests might provide evidence on host relations of all 3 viruses (CV, LSV, LMV) present in flecked commercial Easter lilies. It was learned subsequently that this vector carries LMV infrequently, and also that it may carry both CV and LSV to some plants, and one or the other of these separately to others. The customary disclaimer as to significance of negative evidence is therefore appropriate at this point.

Inoculations to Easter lily seedlings were made parallel with tests on blocks of other possible suscepts, and most of these indicated valid test conditions. Subinoculations to detect CV or LMV were made from the majority of the species exposed, and from all that showed symptoms. No evidence was found in these trials that any of the species enumerated below are susceptible to any of the 3 viruses in question, the positive and possibly significant results having been summarized above (Table 2):—

AMARYLLIDACEAE—*Vallota purpurea* Herb.; ARACEAE—*Philodendron* sp.; COMMELINACEAE—*Commelina coelestis* Willd., *C. nudiflora* L.; CRUCIFERAE—*Brassica rapa* L. (turnip); DIOSCOREACEAE—*Dioscorea alata* L.; IRIDACEAE—*Belamcanda chinensis* (L.) DC.; *Freesia hybrida* Hort., *Iris filifolia* Boiss. (var. Imperator), *I. pallida* Lam., *I. versicolor* L., *Moraea iridioides* L., *Tigridia pavonia* (L. f.) Ker-Gawl., *Tritonia crocata* (L.) Ker-Gawl.; LILIACEAE—*Agapanthus africanus* (L.) Hoffmg., *Allium cepa* L. (var. Yellow Globe), *A. cernuum* Roth, *A. odorum* L., *A. speciosum* Cyr., *Aloe* sp., *Asparagus asparagoides* (L.) W. F. Wight, *A. sprengeri* Regel, *Asphodeline lutea* (L.) Reichb., *Brodiaea uniflora* (Lindl.) Engl., *Camassia leichtlinii* (Baker) S. Wats., *Convallaria majalis* L., *Dracaena sanderiana* Sander, *Erythronium* sp., *Galtonia candicans* Decne., *Haworthia altilinea* Haw., *Hosta plantaginea* Aschers., *Kniphofia tucki* Baker, *Lilium hansoni* Leichtl., *L. henryi* Baker, *L. humboldtii* Roezl & Leichtl., *L. pardalinum* Kellogg, *L. parryi* S. Wats., *L. parvum* Kellogg, *L. sargentiae* Wilson, *L. superbum* L., *Medeola virginica* L., *Muscari polyanthum* Boiss., *Nothoscordum fragrans* (Vent.) Kunth, *Ophiopogon jaburan* (Siebold) Lodd., *Ornithogalum thyrsoides* Jacq., *Sansevieria zeylanica* Willd., *Smilacina racemosa* (L.) Desf., *Smilax* sp., *Tricyrtis hirta* Hook., *Trillium* sp., *Uvularia sessilifolia* L., *Yucca baccata* Torr., *Y. flaccida* Haw.; MUSACEAE—*Musa cavendishii* Lamb., *M. textilis* Née; ZINGIBERACEAE—*Hedychium coronarium* Koen.

Failure to produce CV infection in *Commelina nudiflora* and *Musa cavendishii* is surprising in view of Wellman's (21) success in producing symptoms in both these plants with the celery strain of CV on transfer of *Aphis gossypii.* Inasmuch as parallel trials on Easter lily seedlings were successful, the above results indicate that *Commelina* and *Musa* are not readily affected by lily strains of CV. Natural infection has not been detected in *C. nudiflora* growing as a weed near flecked Easter lilies in Louisiana, nor in bananas occasionally grown for ornament in that State. The commonly heavy infestations of *A. gossypii* on flecked Creole lilies in some sections of Louisiana should produce ample opportunity for infection of the *Commelina* growing adjacent. This weed is reported commonly affected with the celery strain of CV in celery districts of Florida, and is considered an important reservoir of the virus (7).

Lilium sargentiae and *L. superbum,* though not infected in these tests, have been found naturally infected with CV and with LMV (2). It is to be expected that *Aphis gossypii* will carry CV to these and other species of lilies under some conditions, and other vectors of CV, *Macrosiphum solanifolii* and *Myzus persicae,* may also do so.

DESCRIPTION OF LILY-SYMPTOMLESS VIRUS

The evidence developed above is believed adequate to establish the existence of a symptomless constituent of necrotic fleck. This evidence may be summarized as follows: (1) Analysis of naturally occurring necrotic fleck reveals cucumber-mosaic virus (CV) and mottle virus (LMV), but analysis of some experimentally produced necrotic fleck reveals only CV. LMV is, therefore, not an essential constituent of fleck. (2) CV does not produce necrotic fleck in previously virus-free Easter lilies, but does so in certain symptomless commercial Easter lilies. (3) Mechanical transfer from such symptomless Easter lilies to seedlings experimentally infected with CV does not produce necrotic fleck symptoms, but *Aphis gossypii* transfer from the same symptomless source plants does produce fleck. (4) The necrotic-fleck complex has a specific vector, *A. gossypii,* and a well-defined latent period in this vector, whereas CV has several vectors with no such latent period in these.

The symptomless virus LSV, which, in combination with CV, produces necrotic fleck in Easter lilies, does not fall into any of the categories thus far erected for plant viruses. Although the possibility always remains that symptoms may be produced by this virus in some other plant, the tests thus far conducted have demonstrated only one host plant, *Lilium longiflorum.* Inasmuch as this virus is transmitted by an aphid, *Aphis gossypii,* and with difficulty by sap, it shows affinity with the group of viruses placed in *Marmor* (11), and could be placed in the family Marmoraceae if the concept of this family could be broadened to include symptomless forms. Lily-symptomless virus, therefore, is made the type of a new genus *Adelonosus,* which is tentatively assigned to Marmoraceae.

Adelonosus (new genus): Adelos (invisible) + nosos (disease).[3]

Viruses capable of multiplying in living plants, but producing no recognizable symptoms in these, except on interaction with distinct viruses with which they form complexes. Transmitted by aphids, by sap, or by both means.

Type species *Adelonosus lilii*.

Adelonosus lilii (n. sp.).

Common name: Lily-symptomless virus.

Geographic distribution. United States, Japan. Probably coextensive with commercial culture of Easter lily (*Lilium longiflorum*).

Host range: LILIACEAE—*Lilium longiflorum*. No other host established.

Symptoms: No symptoms in pure culture in Easter lily seedlings; necrotic fleck when present together with cucumber-mosaic virus (*Marmor cucumeris* Holmes).

Methods of transfer: By sap with difficulty (3.6 per cent transfer of the complex *Adelonosus lilii* + *Marmor cucumeris*). By *Aphis gossypii* only, among the insect species tested; with a latent period of a few days in this vector. Not through seed.

Properties: Not determined.

Test reaction: Addition of *Adelonosus lilii* to Easter lilies induces necrotic flecking when cucumber-mosaic virus is also present.

DISCUSSION

The conception of a completely symptomless virus is advanced with some reluctance by the writers. It is recognized that a single test plant expressing symptoms would reduce the proposed genus *Adelonosus* to synonymy, possibly with *Marmor*. Such a test species would be of very great value in studies of distribution and properties of this virus. A fairly extensive search for such a test species has therefore been made. Further search will be justifiable if the techniques of manipulating this virus and its vector can be appreciably improved.

The proposed common name, lily-symptomless virus, is acknowledged to be awkward, but the more fitting name, lily-latent virus, has been preempted by McWhorter (13) for a member of the mottle or tulip-breaking group (4), to which Holmes (11) has assigned the name *Marmor mite*.

Lily-symptomless virus (LSV) is far more common in Easter lilies than is cucumber-mosaic virus (CV). This is a surprising fact inasmuch as LSV is known to occur only in Easter lilies and is transmitted in nature only by the melon aphid, whereas CV occurs in many other plants and is transmitted by several other vectors. A smaller discrepancy in range and prevalence might be accounted for by more efficient transfer of LSV by *Aphis gossypii*, as was indicated in some experimental transfers of the necrotic fleck complex, and by evidence that LSV is persistent in its vector. However, the wide difference in prevalence noted suggests that LSV has long been common in most Easter lily varieties, and that CV has more recently become prevalent in these.

The Creole Easter lilies grown for generations in the Delta district of Louisiana are said to have been free from necrotic fleck until recent years, the appearance of the fleck disease dating from the introduction of other Easter lily varieties (Giganteum, Harrisi) for comparison. As late as 1941, when necrotic fleck was prevalent near these centers of introduction, this disease was still absent from isolated plantings of Creole lilies in other Louisiana parishes as well as in Alabama, Florida, South Carolina, and Virginia. The

[3] The writers are indebted to Charles Drechsler for selection of the generic name.

origin of the Creole variety is not known, but it seems probable that lily-symptomless virus was introduced and disseminated with this variety, and that the necrotic fleck disease appeared later after a second separate introduction.

Similarly the Croft and other varieties of Easter lily long cultivated in Oregon are remarkably free of the necrotic fleck disease, but samples tested have proved to be carriers of lily-symptomless virus. The origin of these varieties also is in doubt, but a recent seed origin does not readily account for the prevalence of this virus unless the seedlings were at some time grown near affected Easter lily varieties in a section where *Aphis gossypii* was prevalent.

The fact that Easter lily varieties such as Creole and Croft have, in certain sections, remained free from necrotic fleck for years, shows that cucumber-mosaic virus does not commonly spread from other plants to lilies, inasmuch as only CV is needed to produce necrotic fleck in Easter lilies already carrying LSV. As shown above, the necrotic-fleck complex is readily produced by mechanical addition of CV to such Easter lilies. It may be assumed that any vector of CV that migrates from plants affected with CV can thus produce fleck in such commercial lilies. In other words, though *Aphis gossypii* is the only true vector of the necrotic fleck complex (CV + LSV), any vector of CV might appear to be a vector of necrotic fleck to commercial stocks already affected with LSV. As evidence of such natural synthesis of necrotic fleck is rarely if ever detected in commercial Easter lily culture, the indication is either (1) that there is little natural migration of vectors from other reservoirs of CV to lilies, or (2) that such reservoirs of CV are uncommon in districts of commercial Easter lily culture.

Aphis gossypii is the principal vector of CV in cucurbits (6) as well as in lilies. In our tests this aphid has not been readily transferred from cucumber to Easter lily or vice versa, and presumably does not migrate readily from one to the other in nature. Similarly, free migration from *Echinocystis lobata* Torr. & Gray, the wild cucumber in which this virus is seed-borne, is not to be expected. No information is at hand concerning the freedom of movement of this or other aphid vectors from lily to the recognized perennial hosts of CV, namely *Asclepias syriaca* L., *Phytolacca decandra* L., *Physalis heterophylla* Nees, *P. subglabrata* Mackenzie and Bush, *Nepeta cataria* L. It may be further noted that these potential reservoirs of CV have been found affected only in the vicinity of cucurbit cultures, and that they have not been detected as carriers of CV near Easter lily cultures. It therefore appears, in spite of other possibilities mentioned, that commercial Easter lilies become infected with necrotic fleck chiefly, if not exclusively, from other infected Easter lilies.

Easter lilies produced from seed, and suitably isolated from bulb-perpetuated lilies, remain virus-free. Evidence against seed transmission of CV and LMV is presented elsewhere (4), while evidence against seed carriage of LSV is afforded by data such as those in Sect. A of table 1. Inas-

much as mottle viruses (4) and LSV have very restricted host ranges and, as indicated above, CV does not appear to spread readily to lilies from other sources, there is reason to believe that adequately isolated seedling Easter lilies will remain virus-free indefinitely. Encouraging as they are, these findings are of technical interest only. Present and prospective American producers of Easter lilies are agreed that virus-free culture is impractical. The facts that Easter lily seedlings are too variable for immediate use in forcing, and that rigid isolation must be practiced during the years of selection and increase of new varieties, remove such an undertaking from the field of the commercial producer.

Demonstration of the separate etiology of mottle and necrotic fleck is, on the other hand, of value to such producers who have for practical reasons decided to rogue necrotic fleck but to ignore mottle. As mottle is not an essential constituent of necrotic fleck, mottling can logically be evaluated as a separate disease.

SUMMARY

The necrotic fleck disease of Easter lilies is described, and 2 sub-types, sparse fleck and very sparse fleck, are distinguished in symptom expression, but not in etiology.

The necrotic-fleck virus complex is mechanically transmissible to Easter lily seedlings with difficulty (about 4 per cent successful), but is readily transferred (87 per cent) to commercial Easter lilies.

Cucumber-mosaic virus does not induce necrotic fleck symptoms in previously virus-free Easter lily seedlings, but does induce these symptoms in commercial Easter lilies.

Cucumber-mosaic virus and lily-mottle virus together fail to produce necrotic fleck in previously virus-free Easter lily seedlings.

Only *Aphis gossypii* has been found to transmit necrotic fleck to previously virus-free Easter lily seedlings. *Macrosiphum solanifolii* and *Myzus persicae* have been found to carry cucumber-mosaic virus and lily-mottle virus, but not fleck, to such seedlings. *Aphis fabae, Macrosiphum lilii, Myzus circumflexus,* and *M. convolvuli* were not found to carry any virus from necrotic-flecked Easter lilies to seedlings.

Aphis gossypii transmitted cucumber-mosaic virus from necrotic-flecked Easter lilies to *Calochortus* sp., *Colchicum autumnale, Gloriosa rothschildiana,* and *Fritillaria pudica,* not previously recorded as being susceptible to this virus.

Lily-mottle virus, which commonly accompanies necrotic fleck in Easter lilies, has been found absent from some experimentally produced necrotic fleck in Easter lily, and is therefore not an essential component of necrotic fleck.

A lily-symptomless virus is postulated as an essential component of necrotic fleck, producing necrotic fleck symptoms when present with cucumber-mosaic virus.

Lily-symptomless virus is apparently confined to Easter lily, but the susceptibility of tulip, *Colchicum,* and *Gloriosa,* to this virus remains in doubt. No evidence of susceptibility to this virus was found in other plants tested, namely 56 species representing 40 genera and 9 families, chiefly Monocotyledons.

Lily-symptomless virus occurs in symptomless Creole Easter lilies known as the Norwood strain, in mixture with lily-mottle virus. Sap transfers from the Norwood strain do not produce necrotic fleck in Easter lily seedlings previously infected with cucumber-mosaic virus. Transfers from the same Norwood source by *Aphis gossypii* do produce fleck in such seedlings, thus synthesizing necrotic fleck. Transfers of cucumber-mosaic virus to Norwood Creole, either by sap or by the vector, readily synthesize necrotic fleck.

Lily-symptomless virus passes a latent period of some 4 to 6 days in *Aphis gossypii.* This permits separation of this virus from lily-mottle virus.

A new genus, *Adelonosus,* is created for plant viruses inducing no symptoms, and *A. lilii,* the lily-symptomless virus, is named as the type species.

The distribution and economic significance of necrotic fleck and of the symptomless component are discussed.

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BLACK SCALE: A DISEASE OF EASTER LILY BULBS[1]

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In August, 1937, a specimen of fresh-dug bulbs of easter lily (*Lilium longiflorum*) showing dark-brown to black lesions was brought to this laboratory by D. L. Gill, who collected it in a field in Boothville, Louisiana. A second lot of diseased bulbs from Buras, Louisiana, was brought in a few days later by F. D. Cochran. Both men reported the disease as serious in many lily fields in Plaquemines Parish, and causing considerable concern to the growers.

In October of the same year, the writer visited the lily-growing district to obtain additional material for study and more information on the distribution and importance of the disease. The growers differed in their opinions as to the cause of the disease, some blaming it on poor drainage, others on drought, and others on the fertilizer used, but there was complete unanimity among them that this apparently new disease was extremely destructive, and that it threatened the commercial growing of lily bulbs. Losses as high as 80 per cent were reported by several of the growers. One of the oldest lily growers in the area reported that of a total of 40,000 bulbs he harvested in 1937, only about 7,000 were salable; the rest were diseased.

All the growers interviewed appeared to agree that the disease was new to their area. They stated that a few diseased bulbs were observed in some fields 3–4 years earlier, but that serious losses were not incurred prior to about 1936. Granted that reliance can be placed on these statements of the growers, it is of interest to speculate upon where the disease came from. It is known that on more than one occasion lily bulbs from Bermuda, where the disease is known to occur, have been planted in the lily-growing area of Louisiana, and it may be inferred that the disease was introduced on these bulbs. This, however, is only a speculation.

A study of the disease was undertaken in 1937 and continued for two years. The information obtained from this study showed conclusively that the black scale is caused by a fungus belonging to the genus *Colletotrichum*. Except for a brief abstract (5), publication of these findings has been delayed in the hope that further work might be done on the problem and particularly on efforts to devise some effective control measure. As resumption of the investigations does not appear feasible at present, it has seemed advisable to publish the information obtained with the hope that it may be useful to other investigators.

[1] The disease is known locally under various names, such as "black bulb," "brown bulb," "brown scale," or "black scale." In a preliminary report (5) the writer used the term "brown scale." The name "black scale" has priority, since it has been used to designate what appears to be the same disease on Easter lily bulbs in Bermuda (2, 3, 7, 8), and, for the sake of uniformity, will be used in this publication.

DESCRIPTION OF THE DISEASE

Macroscopic Symptoms. Freshly dug normal bulbs are white to lemon-yellow. In contrast, diseased bulbs show varying degrees of color from brown to nearly black, depending on the number and size of the lesions on the scales (Fig. 1, A). The injury extends to several layers of the scales, the outermost being the most severely affected. Young lesions appear as more or less irregular light-brown areas on the scales. At this stage, the

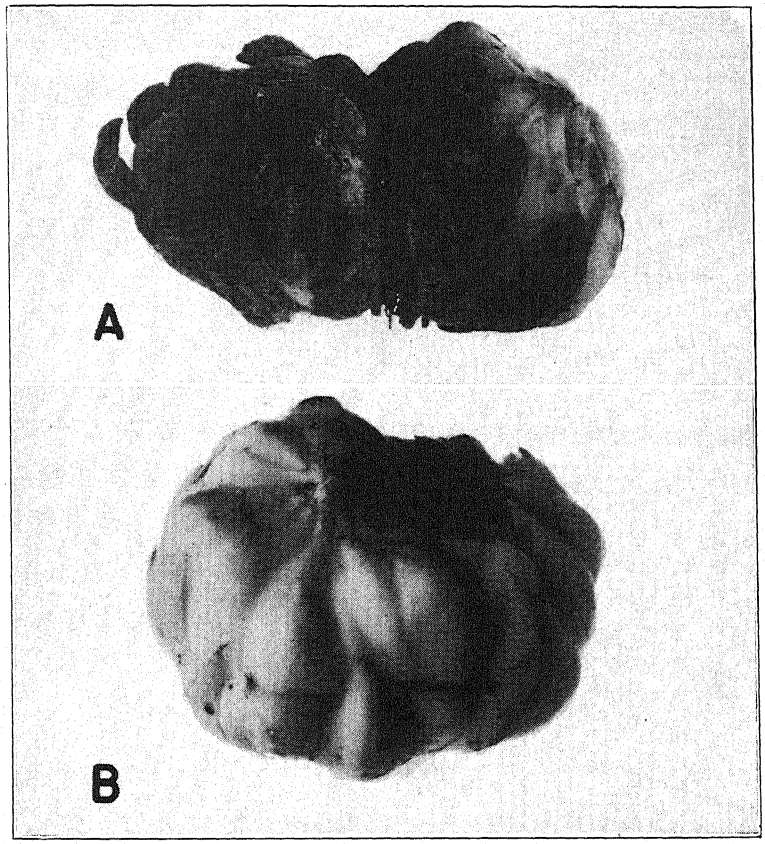


FIG. 1. Black scale of Easter lily. A. Severely diseased bulb. B. Healthy bulb. Approximately natural size.

injury is limited to the epidermal cells. Older lesions are darker-brown and somewhat sunken, due to collapse of the epidermis and one to two layers of the subepidermal cells. Still older lesions are nearly black, and the affected tissues are shrivelled and dried. The lesions are more numerous on the apex half of the scales and, for the most part, occur on the outer (convex) surface, though often are found also on the inner (concave) surface. The plates (stems) and roots appear not to be affected. The core even of severely diseased bulbs appears perfectly normal, and it is known that when diseased

bulbs are planted they grow almost normally and produce flowers. However, because of their unsightly appearance, diseased bulbs are unsalable.

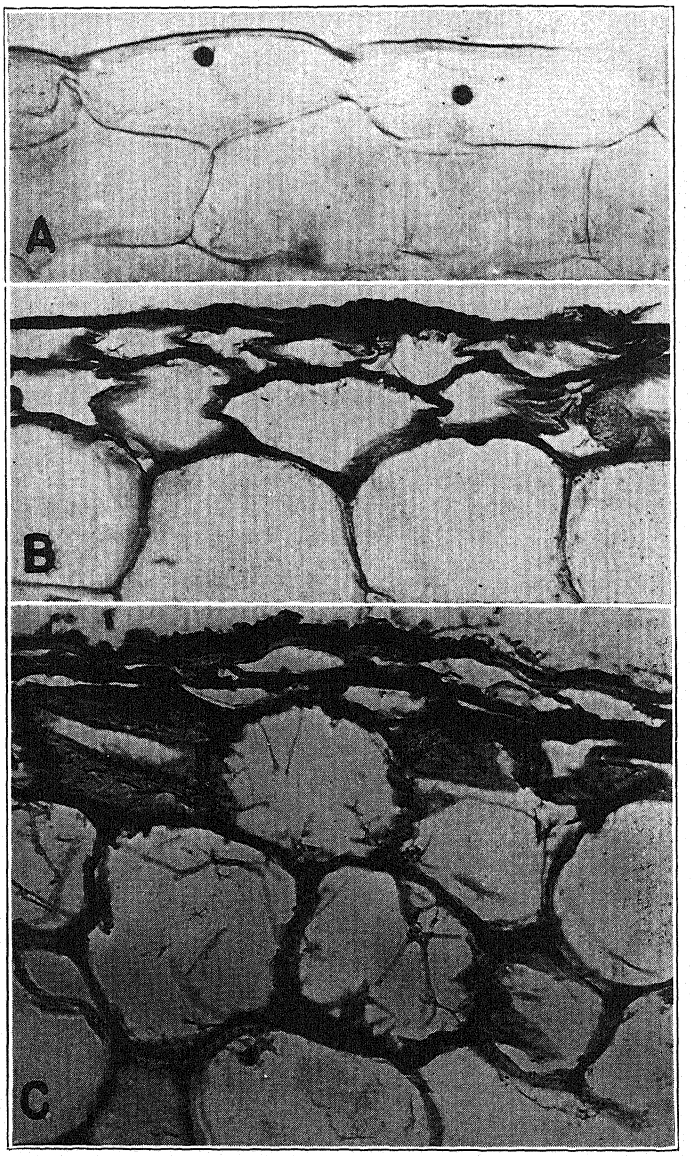


FIG. 2. A. Section through healthy bulb scale showing normal epidermal and first layer of subepidermal cells. B. Section through young lesion showing injury to the epidermis and the first layer of subepidermal cells. C. Section through an older lesion showing injury extending to the fourth subepidermal layer of cortical cells. The epidermis and the first layer of cortical cells have collapsed, and the fungus invasion extends to the fourth layer of subepidermal cells. All × 200.

Microscopic Symptoms: Pathological Histology. The description of the microscopic symptoms is based on examination of hundreds of sections (both

free-hand and paraffin preparations) of diseased scales made through lesions in different stages of development.

In the very young lesions (light-brown spots) the epidermal and the first layer of subepidermal cells are definitely injured, as shown by their brown discoloration, their somewhat flattened shape, and the thickening and flabbiness of their walls (Fig. 2, A, B). At this stage, mycelium occurs sparingly in the tissue, and consists of rather thick hyphae of irregular diameter, occurring between the epidermal and the first layer of subepidermal cells. Mycelium is present on the surface of the lesions at this stage, and it is probable that the fungus kills or injures the tissue in advance of invasion. In later stages (lesions dark-brown and somewhat sunken) the epidermis and the first layer of subepidermal cells are completely collapsed, and mycelium now occurs very abundantly on the surface, beneath the cuticle, and inside the epidermal and first layer of subepidermal cells, and progressively less abundantly in the second, third, and fourth subepidermal layers of cortical cells (Fig. 2, C). No attempt was made to study the disease in old, shrivelled scales because these were usually invaded by secondary organisms, particularly *Fusarium* and *Penicillium*. In general, it appears that in young and moderately old lesions the injury caused by *Colletotrichum* is relatively shallow, limited to the epidermis and the first 2 to 4 layers of subepidermal cells.

FUNGI ASSOCIATED WITH THE DISEASE

Three fungi, species of *Colletotrichum, Fusarium,* and *Penicillium,* were those most commonly isolated when tissue plantings were made from scale lesions. The last-named fungus resembles *P. cyclopium* Westling (4) in culture, but no attempt was made to identify it definitely. It appeared that the longer the bulbs were kept in storage the greater was the percentage of *Fusarium* and *Penicillium* cultures obtained from the tissue plantings and the harder it was to isolate the *Colletotrichum*. On the other hand, when tissue plantings from freshly dug bulbs were made, *Colletotrichum* was isolated very readily and almost exclusively. For example, out of 37 colonies developing from tissue plantings from a specimen of freshly dug bulbs on August 15, 1943, 30 were *Colletotrichum,* 4 *Fusarium,* and 3 unidentified. From another lot of bulbs, which had been in storage for several months, out of 28 colonies growing out of tissue plantings 16 were *Fusarium,* 8 *Peni-*

TABLE 1.—*Fungi isolated from tissue plantings from black scale lesions*

Lot	No. of isolations	*Fusarium*	*Colletotrichum*	*Penicillium*	Other fungi
1	179	60	71	48	0
2	27	12	12	1	2
3	85	31	28	25	1
4	37	4	30	0	3
5	40	28	4	8	0
6	62	14	33	10	5
Total	430	149	178	92	11

cillium, and 4 *Colletotrichum.* The isolations made from 6 different lots of diseased bulbs are summarized in table 1.

PATHOGENICITY

Laboratory Tests

As a preliminary test, the pathogenicity of the fungi isolated was studied in the laboratory. Healthy scales and bulblets were surface-sterilized with 1–1000 $HgCl_2$, washed in sterile water, dried on sterilized filter paper, placed in moist chambers, and inoculated with various isolates of *Fusarium, Penicillium,* and *Colletotrichum* and with combinations of the 3 fungi. The inoculum was placed on punctures made with a flamed needle.

Results. Rotting was caused by all 3 fungi, the *Fusarium* isolates being the most aggressive and the *Penicillium* isolates the least. *Fusarium* and *Penicillium* produced a soft type of rot. In only two instances did *Colletotrichum* isolates produce a more or less superficial, slightly sunken, dry type of lesions, which resembled somewhat the lesions as they occur naturally in the field. In other instances the rot was soft; deep-seated, not much different from that produced by *Fusarium* and *Penicillium.*

Soil Infestation Tests

It was evident that the laboratory method of testing the pathogenicity of these fungi was not satisfactory. In subsequent experiments, the 3 fungi were used, alone or in combination, to infest soil in which healthy bulbs were planted. This method, which approximated field conditions, furnished conclusive evidence that the *Colletotrichum* is the cause of the black scale disease.

Experiment 1. Soil was taken from a field from which about 80 per cent of the bulbs harvested in 1937 were diseased. The soil was divided into two lots, one of which was sterilized by autoclaving for 4 hours; the other was left non-sterilized. Each lot was then subdivided into 12 series. The scheme of the experiment and the results obtained are shown in table 2.

The inoculum was prepared by growing the respective fungi in Mason jars on autoclaved grain (3 parts oats, 1 part wheat). The inoculum was mixed with the soil in the soil-infestation series.

The cottonseed meal (Series C, D, O, and P) was added to the soil in order to test the claims of some of the growers that the disease was either caused by cottonseed meal, or was much more severe when cottonseed meal was used as fertilizer.

The duplicate series (poor drainage), in which glazed porcelain crocks instead of pots were used as containers, were included in order to determine the effect of poor drainage. Several of the growers maintained that the disease was much more severe on poorly drained land. The crocks were not perforated, and the soil in them was flooded 3 times during the course of the experiment to simulate poor drainage conditions. In the "good drainage" series, 7-inch perforated pots were used as containers.

It will be noted by examining the arrangement of the experiment, that the *Fusarium* and *Colletotrichum* inocula (series G, H, S, and T) were used in combination instead of separately, as was done with *Penicillium*. There were several reasons for this. Firstly, the amount of sterilized soil and the number of healthy bulbs at hand when the experiment was being set up were limited and did not allow inclusion of additional series. Secondly, it was suspected that *Penicillium* was the causal organism; so care was taken to use this fungus separately.

TABLE 2.—*Pathogenicity studies. Arrangement and results of experiment 1*

Series and treatment	No. bulbs harvested[a]	No. bulbs diseased	Per cent diseased
Lot I. Non-sterilized soil			
A. Check; non-inoculated, good drainage	7	3	42.8
B. Check; non-inoculated, poor drainage	6	0	0.0
C. Cottonseed meal; good drainage	11	5	45.4
D. Cottonseed meal; poor drainage	8	2	25.0
E. *Penicillium;* good drainage	11	4	36.4
F. *Penicillium;* poor drainage	10	4	40.0
G. *Fusarium* and *Colletotrichum;* good drainage	17	13	76.5
H. *Fusarium* and *Colletotrichum;* poor drainage	7	6	85.7
I. *Fusarium, Colletotrichum* and *Penicillium;* good drainage	17	17	100.0
J. *Fusarium, Colletotrichum* and *Penicillium;* poor drainage	14	11	78.6
K. Diseased bulbs; good drainage	11	11	100.0
L. Diseased bulbs; poor drainage	19	19	100.0
Lot II. Sterilized soil			
M. Check; non-inoculated soil; good drainage	10	1	10.0
N. Check; non-inoculated soil; poor drainage	13	0	0.0
O. Cottonseed meal; good drainage	10	0	0.0
P. Cottonseed meal; poor drainage	2	0	0.0
Q. *Penicillium;* good drainage	9	1	11.1
R. *Penicillium;* poor drainage	15	1	6.6
S. *Fusarium* and *Colletotrichum;* good drainage	13	13	100.0
T. *Fusarium* and *Colletotrichum;* poor drainage	4	1	25.0
U. *Fusarium, Colletotrichum* and *Penicillium;* good drainage	20	20	100.0
V. *Fusarium, Colletotrichum* and *Penicillium;* poor drainage	1	1	100.0
W. Diseased bulbs; good drainage	17	17	100.0
X. Diseased bulbs; poor drainage	7	7	100.0

[a] The number of bulbs harvested was not the same as the number planted because in most cases they multiplied, while in some cases in the poor drainage series soft rot set in and rotted completely some of the bulbs.

The diseased bulbs (series K, L, W, and X) were included in order to see what effect the disease had on their growth and to determine whether or not they would recover. All the other series were planted with healthy bulbs.

The experiment, which was carried on in the greenhouse, was started on October 14, 1937, and the bulbs harvested and examined on June 13, 1938.

Results. The results of experiment I are summarized in table 2. Examining first the first half of the table (Lot I, non-sterilized soil), we see that the disease occurred in all series (except in series B, poor drainage; the

effect of poor drainage is discussed below). This was to be expected, for, as stated in the foregoing, the soil used for the experiment came from a field in which the disease was very severe. Fragments of diseased scales were present, so the soil was well-contaminated. However, even in non-sterilized soil, the series in which *Fusarium* and *Colletotrichum* were used as inoculum (G, H, I, and J) showed a much higher percentage of disease than either the

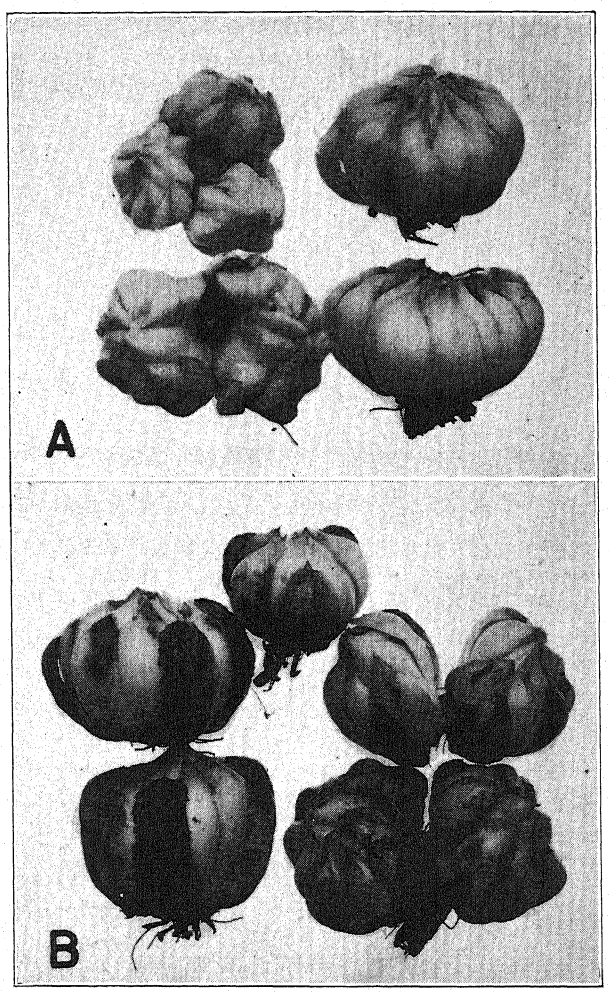


FIG. 3. A. Check. Healthy bulbs grown in sterilized non-inoculated soil. B. Typical black scale symptoms on bulbs grown in soil inoculated with *Colletotrichum lilii* and *Fusarium* spp. Approximately $\frac{1}{2}$ natural size.

check or the *Penicillium* series. The second half of the table (Lot II, sterilized soil) shows definite and clear-cut results. Here, in all series (except T, poor drainage) in which *Fusarium* and *Colletotrichum* were used to inoculate the soil, 100 per cent infection was obtained (Fig. 3). From these results, it was reasonably safe to conclude that either *Fusarium* or *Colletotrichum,* or a combination of the two, was the cause of the black scale.

It should be noted that 1 bulb out of 10 in the check (Series M, Table 2) and 1 bulb in each of the *Penicillium*-inoculated soils (Series Q and R, Table 2) were diseased. This perhaps was due to accidental contamination. Although due precautions were exercised in setting up the experiment, all the containers were located on the same bench in the greenhouse, and chance contamination while watering was possible. It is possible also that some of the bulbs used for planting, although appearing perfectly healthy, might have had incipient infections on some of the scales.

The influence of poor drainage on the disease, under the conditions of this experiment, was interesting. In many instances, both in the sterilized and non-sterilized soil lots (series B, D, J, and T), the percentage of diseased bulbs was lower under poor drainage conditions than in the corresponding series where good drainage was provided. This was because soft rot set in under poor drainage conditions. The soft rot either rotted the bulbs completely or, more often, rotted the outer scales (which would normally show black scale lesions) leaving a clean core. It is not claimed, of course, that the artificial poor drainage conditions provided for in this experiment would correspond to poor drainage conditions in the field.

The addition of cottonseed meal to the soil had no effect on the disease under the conditions of this experiment.

While the diseased bulbs (series K, L, W, and X, Table 2) did not recover, they made good growth and produced flowers. This is in agreement with the experience of the growers.

Experiment 2. Soil was taken from Boothville, Louisiana, from a field in which over 80 per cent of the bulbs harvested in 1938 were diseased. The soil was sterilized by autoclaving for 7 hours. The inocula consisted of 12-day-old cultures of the respective fungi (*Fusarium, Colletotrichum,* and *Penicillium*) on autoclaved oats in Mason jars. In this experiment, not only were the 3 fungi used separately and in combination, but also separate series were run with different isolates of *Fusarium* and *Colletotrichum* picked at random. The experiment was started on November 12, 1938, and the bulbs were harvested and examined on August 14, 1939. Ten 6-inch pots were used for each series, and two bulbs were planted in each pot. The pots were kept in the greenhouse during winter, then removed outdoors in April.

The arrangement of the experiment and the results obtained are shown in table 3.

Results. The results of this experiment show rather definitely that of the 3 fungi found associated with black scale, *Colletotrichum* is responsible for the disease. Each of the 4 isolates of *Colletotrichum,* which had been picked at random from many isolations, as well as a combination of the 4 (Table 3, series I–M), produced the disease. Infection in each case was high, the per cent of diseased bulbs ranging from 85 to 100. Of a total of 146 bulbs harvested from the *Colletotrichum* series, 133, or 91.1 per cent, were diseased. This is considered conclusive evidence that the fungus is the cause of the disease.

TABLE 3.—*Pathogenicity studies. Arrangement and results of experiment 2*

Series	Inoculum	No. bulbs harvested	No. bulbs diseased	Per cent diseased
A	Check; non-inoculated	24	0	0.00
B	*Fusarium* (isolate M31A)	30	1[a]	3.33
C	*Fusarium* (isolate M37B)	25	0	0.00
D	*Fusarium* (isolate M49)	23	1[a]	4.35
E	*Fusarium* (isolate M65)	25	0	0.00
F	*Fusarium* (isolate M75B)	35	0	0.00
G	*Fusarium* (isolate M112)	29	0	0.00
H	*Fusarium* (combination of above isolates)	26	0	0.00
	Total of all *Fusarium* series	193	2	0.01
I	*Colletotrichum* (isolate M32)	29	25	86.20
J	*Colletotrichum* (isolate M60)	27	23	85.18
K	*Colletotrichum* (isolate M80)	27	24	88.88
L	*Colletotrichum* (isolate M117)	30	28	93.33
M	*Colletotrichum* (combination of above isolates)	33	33	100.00
	Total for all *Colletotrichum* series	146	133	91.10
N	*Fusarium* + *Colletotrichum* (all above isolates)	21	19[b]	90.48
O	*Penicillium* (3 isolates M38, M69, and M109)	39	4[c]	10.25
P	*F.*, *C.*, and *P.* (all above isolates)	15	11[b]	73.33

[a] Light infection, 1–2 lesions.
[b] Disease less severe than in series I–M where *Colletotrichum* was used alone.
[c] Doubtful, 1–2 lesions per bulb, rot soft, not typical of black scale.

The *Fusarium*, on the other hand, proved non-pathogenic. Six isolates individually or in combination (Table 3, Series B–H), produced no infection. There were 2 lesions on a scale of 1 bulb in series B and 1 lesion on a bulb in series D. These were assumed to be due to accidental contamination by splattering in watering the pots, or to incipient infection on the bulbs planted. Of a total of 193 bulbs harvested from the soil inoculated with *Fusarium*, 191 were healthy.

Four of 49 bulbs grown in the soil inoculated with *Penicillium* (Table 3, Series O) showed a small amount of disease, but the lesions were not typical of black scale; the rot was rather soft and deep.

Experiment 3. This experiment, set up on November 10, 1939, was essentially a repetition of Experiment 2 with the following modifications:

TABLE 4.—*Pathogenicity studies. Arrangement and results of experiment 3*

Series and treatment	No. of bulbs harvested	No. of bulbs diseased	Per cent diseased
A. Check; sterilized soil, no other treatment	1	0	0.0
B. Check; autoclaved oats mixed with soil	5	0	0.0
C–H. Soil inoculated with *Colletotrichum*	8	7	87.5
I–P. Soil inoculated with *Fusarium*	35	5[a]	14.3
Q. Soil inoculated with *Penicillium*	6	0	0.0
R. Soil contaminated with diseased scales	7	5	71.4

[a] Cultures of both *Fusarium* and *Colletotrichum* were obtained from these bulbs.

1. Soil from Baton Rouge, a mixture of equal parts of heavy black clay and sandy loam, was used. This was sterilized by autoclaving; 2. Additional isolates of *Fusarium* sp. were included as inoculum. These were isolated from diseased bulbs from the previous experiments; 3. One series of 10 pots was included in which the soil was artificially contaminated by incorporating in it fragments of diseased scales.

In all, 18 series of 10 pots each were included. The arrangement of the experiment and the results obtained are shown in table 4.

The bulbs used in this experiment came from a "sick" field, and, although selected as being healthy, they had been in contact with infested soil and diseased bulbs; and it was expected that some, at least, would have incipient infections and also be contaminated with spores. Since these bulbs were large, their outer scales were removed and the clean cores were dipped for 5 minutes in a solution of calcium hypochlorite (33 g. per l. of water) a week before planting. This method of disinfection apparently was not entirely effective, because growth of both *Fusarium* and *Colletotrichum* developed on some of the dipped bulbs when they were placed in moist chambers.

Results. The plants were injured severely by freezing. The pots were on a bench near the door of the greenhouse, and on the night of January 18, during freezing weather, the door blew open and the young plants were severely injured. Soft rot set in following the frost injury, and most of the bulbs rotted completely. The results obtained from the surviving bulbs (Table 4) are, however, in essential agreement with those obtained in experiment 2.

Again a high percentage of disease occurred in the soil inoculated with *Colletotrichum*. That some disease occurred in the *Fusarium* series is not surprising, considering the source of the bulbs used. *Colletotrichum* was isolated from the 5 diseased bulbs of the series. The high incidence of disease in the soil contaminated with fragments of diseased scales (Table 4, R) is noteworthy because it illustrates how the fungus is perpetuated in the soil. Lily scales break easily, and many are left in the soil during digging. Furthermore, scales left in the soil usually do not rot but grow and produce new bulblets. For this reason it is very difficult to clean up a field once it becomes infested.

THE FUNGUS

No critical study concerning the identity of the organisms associated with the black scale disease of Easter lily has been found in the literature. The only references found are in the form of notes or brief reports. It is interesting to note that previous to its appearance in Louisiana (5), all reference to this disease either originated in Bermuda or was about specimens of lilies grown in Bermuda. This would indicate that the disease was not known or had not been recognized in other parts of the world. It should be remembered that most of the earlier reports appeared before the virus diseases of lilies (mosaics and yellow flat) had been identified as separate and distinct

entities; and it is evident from the reports that the symptoms and the possible etiologies of the virus and black scale diseases often were confused.

Halsted (1) found *Vermicularia* and *Phyllosticta* on "all the older or browner leaves" of Easter lily plants submitted to him for examination. Stewart (6), investigating what from his description appears to have been fleck mosaic, reported finding *Vermicularia* and mites on the bulbs, "but by no means constantly and seldom in sufficient numbers to account for the damage." Whetzel (8) stated that the black scale is, in some respects, the most serious disease of lilies in Bermuda, and that "the disease is caused by a fungus, but the identity of the pathogene has not yet been determined." In a personal letter (Dec. 8, 1942) Whetzel has kindly informed the writer that the fungus in question, often obtained from the black lesions on the lily scales, was tentatively identified as *Volutella* or *Vermicularia;* and his belief that the fungus was the cause of the disease had been based on the results of inoculation experiments on healthy scales in moist chambers, but that the results of later experiments (unpublished) had satisfied him that the fungus was probably only secondary, the mosaic being primarily responsible for the disease condition and the results described in his published report. It is apparent that Whetzel was not dealing with a single disease; his description of the trouble was obviously a composite picture of the symptoms of two diseases, black scale and fleck mosaic. Ogilvie (2) stated that *Volutella* sp. was the cause of black scale, although in a later report (3) he expressed a different view: "Work on the spotting of the bulbs common in certain soils ('black scale') again showed that this condition is a physiological one due to small points of asphyxiation taking place in heavy or water-logged soils, and cannot be classed as a disease. Such bulbs produce healthy plants. The Volutella sometimes found on the spots is purely secondary."

Waterston (7) named *Colletotrichum gloeosporioides* as the probable cause of black scale, although apparently he had a doubt regarding the identity of the fungus. He stated: "Black scale, ? *Colletotrichum gloeosporioides* Penz. has been provisionally named the fungus responsible for this condition in the bulb and for a premature die-back of the plant in the field."

In a preliminary report (5) the fungus responsible for the disease in Louisiana was tentatively identified as *Vermicularia.* Later, a more critical examination showed that the fungus was definitely not a *Vermicularia* and that it fitted well in the genus *Colletotrichum.* The fruiting body was found to originate beneath the cuticle as a small stroma from which arise the setae which rupture the cuticle. Conidia are produced after the cuticle has been ruptured and the acervulus has been formed (Fig. 4). At no time during its development has the fruiting body a cover of fungus origin.

The fungus grows well in culture. The colonies are at first white, with much aerial growth turning gradually gray and finally nearly black on top with a pinkish tinge in the substratum. In old cultures, the aerial mycelium becomes suppressed and the surface of the colony may become smooth, black,

glistening, somewhat leathery. Young isolates usually produce a great profusion of acervuli on the surface of the agar, with thick, raised masses of conidia through which the setae protrude. Macroscopically, the acervuli at this stage resemble pycnidia. After the isolates have been kept in culture for some time, they usually lose their ability to form acervuli, but they still continue to produce conidia loosely on the mycelium in abundance.

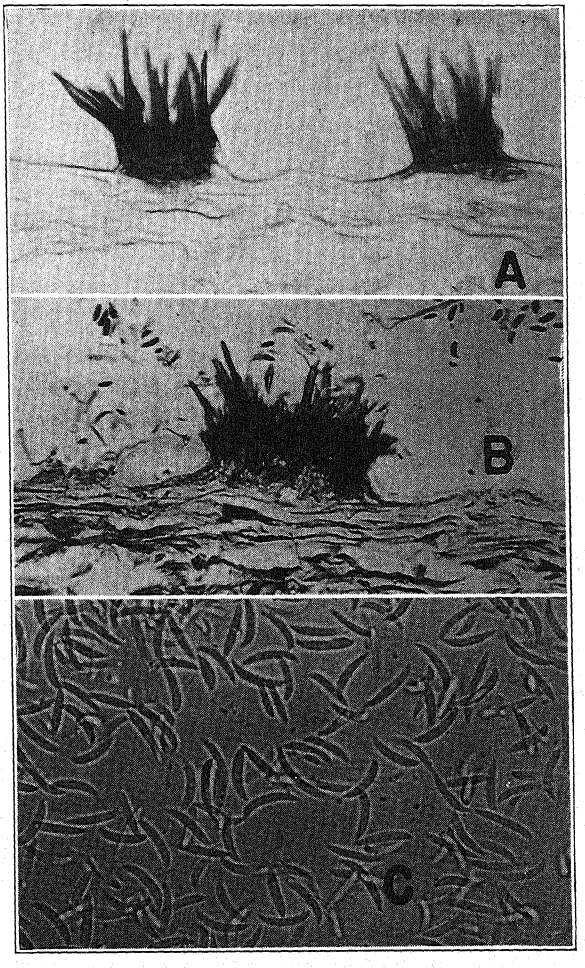


FIG. 4. *Colletotrichum* **lilii**, sp. nov. A. Young acervuli with setae but no conidia. ×200. B. Mature acervulus. ×200. C. Conidia from a 10-day-old culture. ×400.

No description of a *Colletotrichum* on lilies has been found in the literature that fits the causal fungus of the black-scale disease. It is possible, of course, that this fungus has been described on another host, but in view of our present limited knowledge of the pathogenic specificity of many fungi, it is considered advisable to describe it as a new species, for which the following name is proposed.

TECHNICAL DESCRIPTION

Colletotrichum lilii, sp. nov.

Acervuli small, mostly gregarious, arising subcuticularly, on a small stroma, dark brown, densely setose throughout, 43.0–122 μ, ave. 67.2 μ; setae dark brown, rigid, straight, continuous or septate, 29.7–72.6 × 4.0–5.3 μ, average 45.7 × 4.9 μ; conidiophores hyaline, subconical, short, about 10 × 4.5 μ; conidia hyaline, falcate, continuous, subacute, usually vacuolate, 13.2–23.0 × 3.6–4.9 μ, ave. 18.0 × 3.7 μ.

Hab. on scales of bulbs of *Lilium longiflorum*.

Type locality: Plaquemines Parish, Louisiana.

Type specimens: Type specimens, consisting of dried scales, dried agar cultures, and slides, have been deposited in the herbarium of the Botany Department, Louisiana State University (accession No. 4709), and also in the Mycological Collections, Bureau of Plant Industry, Washington, D. C.

ATTEMPTS TO CONTROL THE DISEASE

A few exploratory tests were made over a 2-year period (1938–1939) in an effort to obtain information that might point the way toward effective measures of control. These efforts were directed toward 2 separate objectives, viz: (a) to disinfest the soil in "sick" fields so that healthy bulbs might safely be planted in it, and (b) to disinfect diseased bulbs so that these might be planted in disease-free soil without the danger of spreading the infection to new fields. Unfortunately, all the experiments gave negative results. Some of the treatments were merely ineffective against the disease, but without any harmful effects on the bulbs, while others, in addition to being ineffective, caused varying degrees of injury to the bulbs. The various treatments tried are listed and discussed briefly, even though the results were negative, in the hope that they may serve as a guide in future work on control of this serious disease.

Soil Treatments

The soil treatments were made in 1938 under field conditions in Boothville, Louisiana. These included, (1) chloropicrin at the rate of 2 cc. and 3 cc. per sq. ft. of soil, (2) sulphur ("Toro" brand) at the rates of 700 and 1000 lb. per acre, (3) basic copper sulphate ("Copper Spray 34") at the rate of 1.75 lb. per 25-foot row, and (4) calcium hypochlorite at the rate of 1 lb. per 25-foot row. The sulphur was broadcast and mixed with the soil before the ridges (rows) were made up. The basic copper sulphate and the calcium hypochlorite were applied as narrow bands in the rows and mixed thoroughly with the soil just before planting. All treatments were replicated 3 times in 3 different fields. The soil of fields I and II was of the same type, known locally as "coffee grounds" soil. It was reclaimed land, composed of heavy alluvial soil containing considerable peat-like, imperfectly decomposed vegetable matter. Both fields had a similar history in that both had been planted with lilies the previous year and in both fields practically 100 per cent of the bulbs harvested were diseased. The soil of field III was of the best in that area. It was black, heavy, alluvial with its organic matter well decomposed. This field also was planted with lilies the previous year, and about 50 per cent of the bulbs harvested were diseased, according to the owner. Healthy bulbs were planted in all plots.

Results. All treatments proved ineffective. It is true that in one field all treatments gave significantly higher percentages of marketable bulbs (healthy and slightly diseased) but these differences were not maintained in the other replications. The sulphur and chloropicrin had no effect, harmful or beneficial, on the bulbs. The basic copper sulphate and the calcium hypochlorite, on the other hand, at the rates used, proved somewhat toxic. This toxicity was manifested as delayed emergence, poorer stands, and weaker growth of the plants compared with those of the check and other plots.

The conditions of the experiments were definitely inimical for a fair test of the efficacy of chloropicrin. The soil in the test plots was very heavy, and, in spite of repeated discing and harrowing, large clods were left on the surface that could not be reached by the fumes. No cover was used, and no facilities were available for sprinkling the surface with water to form a seal. Under more favorable conditions, it is possible that chloropicrin might prove effective, for this chemical has been found by many investigators to be a potent soil disinfestant.

TABLE 5.—*Comparison of the corresponding check plots of fields I (diseased scales not removed from the soil) and II (diseased scales removed)*

Field	Healthy bulbs	Lightly diseased bulbs	Severely diseased bulbs	Marketable (healthy plus lightly diseased bulbs)
	Per cent	*Per cent*	*Per cent*	*Per cent*
I	16.3	24.0	59.7	40.2
II	26.4	44.2	29.4	70.6

A noteworthy result of this experiment was the difference in the amount of disease between corresponding plots of like treatments in fields I and II. These two fields, located about one-half mile apart, were as nearly alike as possible. They were of the same type of soil, both were planted to lilies the previous year, and in both approximately 100 per cent of the bulbs harvested in 1938 were diseased. There was, however, this one important difference: In field II, an effort was made to remove from the soil as many as possible of the loose scales, and fragments of scales, that were left from the previous harvest; in field I, no such effort was made, and the soil was thoroughly contaminated with diseased scales. A comparison of the amount of disease in the check plots of fields I and II is shown in table 5. It may be seen that a significantly lower percentage of severely diseased bulbs was obtained from field II than from field I. Differences of about the same magnitude were obtained by comparing other corresponding plots of the two fields. It is believed that less disease occurred in field II simply because most of the infected scales had been removed from the soil, thus lessening the amount of inoculum. If this assumption be correct, then it would seem that cultural practices combining sanitation and rotation would offer a practical way of reducing, if not completely controlling, the disease.

Bulb Treatments

Since infection is relatively shallow, *i.e.*, the fungus does not penetrate deeply into the tissue, theoretically, it might be possible to find some fungicide that would kill the fungus without causing much injury to the bulb. Actually, as brought out by the results of a limited number of trials with various chemicals and combination of chemicals, this does not appear to be so easy. In no case was control obtained, and, in addition, the treatments showed varying degrees of injury to the bulbs. It is admitted, of course, that only a limited number of treatments have been tried, and that neither the list of fungicides, nor the dosages and exposures have been exhausted. It is still possible that an effective treatment may be found, but the results thus far obtained indicate that this line of attack is not particularly promising.

Typically diseased bulbs were secured. The outer, shrivelled scales were removed leaving only the inner live scales, which, however, still contained lesions of various sizes. The bulbs were treated and planted in duplicate sets in sterilized and nonsterilized soil in pots in the greenhouse. Records were kept on the effect of the treatment on germination and subsequent growth. Then, in late summer, the bulbs were dug and examined for presence or absence of disease. These tests were made in 1938 and 1939, as follows:

A. Borax dip, 7.5% solution, 6 minutes at 40° C.

B. Mercuric chloride 1–1000 in 50% alcohol + 1% acetic acid, 3 minutes.

C. Same as B but dipped for 6 minutes.

D. Alcohol (70%) 1 liter; acetic acid 15 cc., mercuric chloride 2 g. Dipped for 10 minutes.

E. Acetic acid 3%, brilliant green 1–20,000, in 50% alcohol, 15 minutes.

F. Bulbs dusted with basic copper sulphate (34% Cu), excess dust screened off.

G. Basic copper sulphate (34% Cu) mixed with the soil at the rate of 150 g. per 50 lb. of soil.

H. Same as in G, but at the rate of 200 g. per 50 lb. of soil.

I. Calcium hypochlorite mixed with the soil at the rate of 200 g. per 50 lb. of soil immediately before planting.

Results. None of the treatments gave any control. The bulbs harvested from all treatments were as severely diseased as those of the checks. In addition, the following toxic effects were noted:

1. Borax. Tip-burn and chlorosis of the lower leaves of the plants after emergence; otherwise growth was normal.

2. The alcohol-acetic acid-mercuric chloride dip for 3 minutes (B) caused no apparent injury.

3. All the rest of the treatments caused injury, manifest in failure of some of the bulbs to emerge and in delayed emergence and weaker, poorer growth of the plants in comparison with those of the checks.

In connection with another disease (mosaic), lily bulbs were treated with hot water, 52° C. for 30 minutes. The bulbs of this lot were not affected with black scale, so no information was obtained on the effect of hot-water treatment on this disease. However, the 30-minute bath at 52° C. was definitely injurious to the bulbs. Some did not grow at all, while others made a decidedly poorer growth than the checks. It is likely, however, that a favorable combination of temperature and period of exposure may be found that will be effective in disinfecting the bulbs without appreciable injury.

SUMMARY

A serious disease of Easter lily, which causes dark-brown to black lesions on the scales of the bulbs in the ground, is described under the name of "Black Scale."

Pathogenicity studies have shown that the cause of the disease is a fungus that fits well in the genus *Colletotrichum*. It is considered to be an undescribed species, and is described as one new to science under the name of *C.* **lilii** sp. nov.

A limited number of experiments consisting of chemical treatments of the diseased bulbs or of infested soil, have been tried. All gave negative results.

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STRIPE REACTION OF SPRING BARLEY VARIETIES[1, 2]

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The stripe disease of barley, caused by *Helminthosporium gramineum* Rabh., has been under observation by workers in the United States and foreign countries for a period of years. When Oderbrucker varieties of barley were grown widely in Wisconsin, stripe often caused yield losses; but with wide-spread usage of Wisconsin Barbless the disease has been less prevalent. Even though stripe has not caused serious yield reductions in recent years, the disease has been given consideration in the general barley-breeding program at the Wisconsin Agricultural Experiment Station. Emphasis has been placed on finding varieties with good agronomic type as well as resistance to stripe and other diseases. Such varieties, used as parental stocks, would likely reduce the breeding problems. New varieties should carry a practical type of resistance, so as to prevent natural stripe epidemics.

EARLIER WORK

Earlier work concerning barley varietal reaction to stripe was carried out by Christensen and Graham (2), DeHaan (3), Fuchs (4), Genau (5), Isenbeck (6), Johnson (7), Majdrakoff (8), Shands (9), Shands *et al.* (11), Stelzner (12), Winklemann (14), and Yu and Hwang (15). The methods of testing varied considerably both in technique and in effectiveness. Shands (9) gave a brief review of the methods of inoculation used. Yu and Hwang (15), used floral inoculation, while the others used the mycelium method for seedling inoculation, except for Stelzner (12) who used a conidial suspension and a partial evacuation method for inoculation of the seed.

In Germany, Isenbeck (6) tested a number of spring and winter barley varieties, using both the floral inoculation and inoculation of germinating kernels. Some of his infections were quite high, but results for individual varieties were not given. Yu and Hwang (15), working in China, have reported detailed readings on the stripe reaction of a large number of varieties. Using conidial inoculation of the flowers between the milky and green-mature stages, their infections averaged 5.4 per cent for the 190 varieties. Their highest 3-year average infection was 51 per cent for Kumflide (C.I. 730). Their local susceptible variety averaged 37.5 per cent for the 3 years. Because a large proportion of the varieties had very low percentages of striped plants, and also because of specialization in *Helminthosporium gramineum,* it is doubtful whether their results could be safely applied under North American conditions. Had flowers been inoculated earlier

[1] These studies were supported in part by funds provided by the Wisconsin Alumni Research Foundation.

[2] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

more infection might have resulted. Christensen and Graham (2) tested a smaller number of varieties and obtained rather high percentages of infection, especially with cultural races 33, 34, and 42. Svansota, Manchuria, Velvet, Peatland, and Alpha were relatively susceptible: while Kama-ore (C.I. 694), Trebi (C.I. 936), and the Black Hulless selection were resistant. Stelzner (12), using the conidial-suspension and partial-vacuum techniques of Fuchs (4) and Majdrakoff (8), found some varieties more resistant than others, but his infection percentages were lower than those of several previous workers. Suneson and Santoni (13) florally inoculated a susceptible male-sterile stock that had been previously pollinated with pollen from certain varieties under observation. In this way they compared varieties on the basis of F_1-progeny responses.

Isenbeck (6) tested the reaction of several barley varieties to different collections of the stripe fungus as did Christensen and Graham (2). In these papers it was shown that certain cultures of *Helminthosporium gramineum* are selectively pathogenic and that certain varieties are generally more resistant than others. *H. gramineum* is not so highly specialized as the rusts and smuts, but this specialization should not be overlooked in testing for varietal reaction. However, for practical purposes a few well-selected, highly pathogenic cultures could be used to get a fairly reliable estimate of the reaction of a variety.

METHODS AND MATERIALS

Inoculation

The method of inoculation for varietal tests reported in this paper has been briefly described by Arny and Shands (1). This method is concerned with mycelial inoculation of germinating kernels, and is as follows: After adding sterile water, fine aggregations of mycelium of *Helminthosporium gramineum* are scraped from a 7-day-old slant culture. Two cc. of this suspension are added to each 125-ml. Erlenmeyer flask previously prepared by mixing 15 g. of wheat and 15 cc. of water and autoclaving 45 minutes. When fungus growth on wheat is 5 days old, 100 kernels of the barley variety or selection being tested are surface-treated with 70 per cent alcohol, rinsed in water, and placed in the flask. The contents of the flask are shaken immediately to mix kernels with inoculum and incubated 4 days at room temperature (20–24° C.). Flasks are shaken daily to prevent matting and clumping caused by mycelium and root growth. The number of kernels per test can be varied, although 100 were used for the most part in the varietal tests, and incubation temperature can be lowered if a longer time is used. The entire contents of the flasks are planted in the soil. With this method about 50 per cent of the kernels produce plants in the field, while about 80 per cent produce plants in the greenhouse. In susceptible varieties about 75 per cent stripe infection develops in 6 or 7 weeks time, some infection showing as early as 2 weeks after seeding.

Varieties Tested

A large number of the varieties tested came from the C.I. collection.[3] The seed was originally supplied by R. G. Shands. Previous to the stripe test most of the varieties used had been found resistant in inoculation trials with the 2 sporidium-forming smuts. Also tested for stripe response were selections from the barley-breeding program in progress at the Wisconsin Agricultural Experiment Station. A large number of these hybrid selections do not carry high resistance to the smuts of barley. For the most part selection numbers preceded by "H" were from R. G. Shands.

Cultures Used

The cultures of *Helminthosporium gramineum* were of hyphal-tip origin and date to 1932, 1933, and about 1934 for C–1, 17–1, and 45–2, respectively. These cultures appeared to be stable and pathogenic on a large number of varieties. Culture 17–1 was from a conidium produced on a striped Oderbrucker plant in a row that had been inoculated with culture C–1. Atlas (C.I. 4118) is a variety known to have stripe-infected plants under field conditions in California, and, yet, had no striped plants in 4 artificial inoculation tests reported in this paper. This suggests that some of the varieties, appearing highly resistant under the set of conditions used, may be susceptible in other areas where different conditions of infection might exist. Before assuming a varietal response an investigator should artificially inoculate the varieties in which he is interested, using local cultures and environment. Even though specialization is known to exist, the varietal responses reported herein are thought to be fairly reliable under a wide range of conditions.

RESULTS

Tests have been carried out mostly in the field from 1935 to 1942, although a few were made in the greenhouse. The results of 1936, 1937, and 1939 had lower infection, thereby being less reliable; and because of this only a small part of the data was included. Tests with less than 10 plants were omitted. If more than 50 per cent stripe was found, the variety may not have been tested further; when less than 50 per cent was observed, the variety was tested one or more additional times. In general each variety was tested with at least 2 cultures in any one year.

The results of the several years' tests are combined in tables 1 and 2. Where possible, the C.I. number and the name of each variety are given with the number of tests, the total number of plants observed, low and high per cent infected plants, and the average per cent of infected plants on a weighted basis. The weighted average is determined by use of the total plants in all tests of an individual variety. All of the varieties used in the test are of the spring type, although some are late in heading. Most of the varieties are 6-rowed, rough-awned, with white adhering hulls, and white or

[3] A world collection of barley varieties maintained by the Division of Cereal Crops and Diseases, U. S. Dept. Agriculture.

TABLE 1.—*Stripe reaction of barley varieties when artificially inoculated*

C.I. No.	Name and description[a]	Tests	Total plants	Stripe infected plants		
				Low	High	Weighted average
		No.	*No.*	*Per cent*	*Per cent*	*Per cent*
241	Manchuria	3	117	25	75	42
276	Coast	3	108	13	82	59
531	Hannchen 2	2	136	4	11	7
595	Nepal H, N	2	94	16	16	16
652	Poda	2	64	67	68	68
663	Chile Common	3	114	39	87	65
668	Abyssinian D, P	4	130	22	72	47
918	Korsbyg	7	223	0	9	2
920	White Gatami	8	457	0	2	1
923	Lion (Leiorrhynchum) Bk, S	2	129	9	16	12
934	Odessa	6	270	0	76	23
936	Trebi	9	513	0	25	5
940	Oderbrucker	4	242	58	91	81
967	Jet 2, Bk, N	1	15	.....	.....	93
971	Daniels	2	68	37	96	60
972	Luth	3	147	8	72	46
995	Scarab	4	179	4	30	22
998	Iris N	2	71	89	100	94
1015	Ederle N	2	108	4	7	5
1016	Kwan	3	92	3	35	16
1017	Monte Cristo	3	172	20	39	35
1021	Weider	3	133	0	19	9
1023	Czech	2	137	8	26	16
1024	Quinn	2	137	0	2	1
1027	Black Hulless P, N	6	225	9	37	27
1037	Coventry	2	151	0	0	0
1076	Barquis	2	83	53	59	57
1087	Nani Tal	6	307	0	4	1
1090	Crypt	2	131	25	32	28
1094	Crocket	2	97	19	25	22
1110	Chalet 2	2	138	0	0	0
1111	Chevron	9	458	0	21	7
1118	Featherston	2	149	67	76	71
1137	Oderbrucker	1	22	.....	.....	95
1223	Abyssinian	1	31	.....	.....	84
1243	Abyssinian A	3	53	50	82	70
1248	Excelsior P, N	2	118	87	92	89
1257	Bolivia	2	104	49	66	54
1263	Fleche	2	41	19	28	24
1296	Kitchin D, P	5	203	41	71	62
1312	Lompoc N	2	92	0	0	0
1315	Kusan	2	83	85	97	90
1326	Childs 2, H	2	94	19	43	30
1330	Pannier	2	82	91	93	92
1355	Jusborne S	3	192	35	61	38

[a] All are white or blue, 6-rowed, rough-awned, and have adhering lemma and palea, except where other characteristics are indicated according to the following key:

2 = 2-rowed
I = intermedium
D = deficiens
N = naked kernels
A = Abyssinian intermediate
S = smooth awned
SS = semi-smooth awned
Bk = black
P = purple
H = hooded

Bk and P refer to black or purple color in lemma and palea of threshed grain of hulled barleys and to the color of the caryopsis in naked barley.

TABLE 1.—(*Continued*)

C.I. No.	Name and description[a]	Tests	Total plants	Stripe infected plants		
				Low	High	Weighted average
		No.	*No.*	*Per cent*	*Per cent*	*Per cent*
1370	Spain P, N	2	31	15	28	23
1387	Cape (Transvaal Early)	4	238	1	15	5
1409	McFadden S	1	43	.....	.....	98
1413	Gatami Bk	4	178	0	0	0
1417	Squarehead	1	42	.....	.....	98
1435	Manchuria	1	46	.....	.....	85
1466	Kutan 2	2	58	0	8	4
1468	Burat	2	66	24	50	35
1470	O.A.C. 21	5	243	68	98	83
1473	Manchuria	2	78	44	82	58
1511	Golden Queen	2	59	50	95	80
1517	Oderbrucker	2	68	73	91	79
1529	do. (Wis. Ped. 6)	2	161	53	83	67
1551	Albert	1	47	.....	.....	96
1557	Claude	2	69	65	79	71
1577	Aker	2	62	55	68	60
1603	Ireland A	2	31	20	64	36
1613	Cross	3	108	0	9	2
1614	Fleche	3	143	15	52	36
1615	Ishtar	2	90	0	0	0
1621	Mars	2	96	21	53	37
1622	Osiris	2	105	13	32	23
1692	Mokar	9	469	29	84	69
1698	Reed Triumph	3	206	68	94	76
1969	Chinese Black Bk	4	181	0	0	0
2040	Canada Winter	3	69	0	0	0
2080	White Smyrna 2	3	70	0	17	7
2135	Golden Drop 2	2	128	20	33	26
2201	Eagle	2	52	67	100	75
2203	Boehme's Beardless H	1	30	.....	.....	67
2208	Ethiops Bk, H, N	1	12	.....	.....	92
2213	Nudi haxtoni I, N	2	64	0	0	0
2214	Nudi mortoni I, Bk, N	2	33	12	87	52
2215	Cornutum I, H, N	2	79	0	0	0
2226	Steudelii D, Bk	3	123	10	37	25
2242	Purple Nepal P, H, N	2	36	0	39	20
2244	Surprise	2	66	48	100	64
2253	Kamet Mugi N	2	54	0	0	0
2254	Huwan I	4	129	0	12	3
2259	Manchuria	2	69	67	92	76
2261	Pusa N	2	33	0	0	0
2276	Gatami Bk	4	151	0	2	1
2277	Black Hulless P, N	2	66	29	29	29
2280	Arlington Awnless I	6	206	0	3	1
2282	Pannier	2	33	53	83	69
2284	Black Abyssinian A, Bk	3	122	0	7	2
2286	Nudi haxtoni I, N	2	76	0	0	0
2292	Intermediate I	2	60	45	59	50
2318	Kharsila N	2	41	4	13	7
2319	India N	2	46	0	0	0
2330	Manchuria (Minn. 184)	3	101	66	100	78
2338	Nigrum Bk	2	77	0	3	1
2352	Nudi haxtoni I, N	2	80	0	0	0
2368	Frozoff	1	24	.....	.....	79
2371	.....	2	61	79	100	85

TABLE 1.—(*Continued*)

C.I. No.	Name and description[a]	Tests	Total plants	Stripe infected plants		
				Low	High	Weighted average
		No.	*No.*	*Per cent*	*Per cent*	*Per cent*
2381	..... A	2	74	8	17	12
2382	..... A	2	55	0	17	11
2421	.....	2	73	72	96	81
2432	Rakoff	2	72	8	19	14
2433	Duplex	3	124	25	58	43
2437	Newbly 2	2	99	0	2	1
2448	Himalaya N	2	65	23	43	34
2454	Pegan	2	58	0	7	4
2467	Sampan	4	202	2	13	5
2481	Hanful	2	60	25	58	38
2483	Modia	2	65	0	0	0
2492	Cross	4	235	0	3	1
2494	Manchuria	2	59	73	91	79
2500	Verigat Bk, H	2	37	33	70	43
2505	Englawnless 2	2	30	0	20	7
2509	Englawnless 2	2	51	0	9	2
2511	Plumage 2	2	96	23	36	29
2514	Abyssinian Intermediate A	2	31	16	50	29
2519	.....	2	99	0	0	0
2524	.....	3	141	0	4	3
2542	.....	3	146	2	3	3
2543	.....	2	101	0	2	1
2553	Cap Rouge	2	53	77	90	86
2561	..... S	2	86	38	54	47
2570	..... S	2	67	24	40	31
2572	..... S	2	83	64	64	64
2573	..... S	2	98	0	2	1
2577	..... S	2	92	7	8	8
2589	..... S	2	93	12	14	13
2591	..... S	2	80	15	38	27
2606	..... SS	2	63	0	0	0
2607	 I	2	47	46	52	49
2608	Odessa	2	105	6	33	20
2610	Manchuria	2	104	26	52	40
2618	do.	2	105	60	70	64
2621	Featherston	2	96	75	82	79
2628	Chicago	2	82	71	79	74
2632	.....	2	96	70	95	80
2750	Canadian Lake Shore	2	95	44	57	50
2947	Manchuria (N.D. 2121)	7	377	69	93	86
2982	South Russian	2	125	29	68	47
3124–1	..... D	1	16	.....	.....	63
3187	Regressive 2	2	75	26	41	32
3196	Ehrhardt Frederiksens	2	36	58	75	64
3206–1	.....	2	59	6	26	15
3206–3	.....	2	79	46	63	56
3208–4	.....	4	104	43	93	71
3210–2	.....	6	145	33	73	54
3211–2	.....	1	17	.....	.....	53
3230	..... N	2	62	0	13	7
3245	.....	2	100	30	43	36
3393–2	Bonfarik	2	145	7	7	7
3402	Gujarkhan	4	91	0	6	2
3403	Lyallpur E	3	69	0	24	13
3404	Ludhiana	4	142	13	39	27

TABLE 1.—(*Continued*)

C.I. No.	Name and description[a]	Tests	Total plants	Stripe infected plants		
				Low	High	Weighted average
		No.	*No.*	*Per cent*	*Per cent*	*Per cent*
3408	Delhi	2	70	33	38	35
3410	.....	5	222	0	11	5
3443	India Guzerat	5	225	6	34	19
3485	 2	2	35	0	0	0
3508	.....	2	42	13	21	17
3509	..... N	2	38	26	67	42
3510	..... N	2	46	41	65	50
3530–1	.....	2	74	0	4	1
3552	.....	4	183	0	15	8
3553	.....	4	99	9	44	26
3554	.....	4	240	6	60	19
3555	.....	2	76	2	28	13
3557	.....	2	59	41	85	56
3558	.....	4	191	12	41	19
3559	.....	4	192	0	0	0
3560	.....	2	59	52	78	66
3562	.....	2	61	23	37	31
3570	.....	2	71	2	8	4
3573	 2	3	91	0	7	3
3581	.....	2	80	24	60	38
3582	.....	4	161	12	78	43
3587	.....	2	69	14	44	28
3588	.....	2	72	3	6	4
3614	..... 2, SS	3	106	0	5	1
3617	.....	2	75	21	25	22
3623	 2	3	74	11	50	27
3634	.....	4	151	0	4	1
3635	.....	2	81	0	0	0
3650	.....	2	80	11	15	13
3657	.....	2	80	5	8	7
3659	.....	2	93	0	2	1
3668	 2	3	102	0	14	4
3683	.....	2	72	17	39	25
3694	 2	3	105	0	0	0
3704	 2	3	112	5	46	29
3721	.....	2	66	22	62	43
3724	.....	2	90	0	3	1
3728	.....	4	199	0	15	9
3745	.....	4	167	2	21	8
3746	.....	2	94	11	26	17
3776	.....	4	129	32	72	45
3778	.....	3	118	17	45	25
3780	.....	2	64	0	27	9
3792	.....	2	93	14	86	47
3794	.....	2	81	5	26	15
3797	.....	2	72	2	4	3
3808	.....	4	201	5	17	11
3810	.....	4	182	2	40	14
3816	.....	4	149	7	23	14
3895–1	Revil	4	176	0	0	0
3896–1	.....	4	167	0	11	5
3897–2	.....	4	153	0	3	1
3902–1	Morocco	5	220	0	3	1
3903	.....	5	231	0	50	21
3971–1	.....	2	56	0	12	5

TABLE 1.—(*Continued*)

C.I. No.	Name and description[a]	Tests	Total plants	Stripe infected plants		
				Low	High	Weighted average
		No.	*No.*	*Per cent*	*Per cent*	*Per cent*
4041–2	.....	2	75	3	14	9
4118	Atlas	4	92	0	0	0
4184	.....	2	33	0	0	0
4200–1	.....	2	60	0	0	0
4252	Velvet S	8	419	28	73	45
4289	July	5	111	3	53	43
4425–1	.....	2	98	75	83	80
4428–1	.....	2	108	56	93	76
4500–1	.....	2	94	95	96	96
4502–2	.....	2	105	53	96	72
4559	.....	2	68	3	3	19
4559–1	.....	4	215	26	38	59
4561	.....	4	238	9	50	27
4576–1	.....	2	112	87	94	90
4577	Glabron S	4	235	16	44	30
4577–1	..... S	2	137	15	25	20
4585	Vance 2, S	2	120	0	0	0
4622	.....	4	236	0	2	1
4623	.....	4	191	0	0	0
4637	.....	2	138	36	39	37
4666	Oderbrucker (Wis. Ped. 5–1)	91	4217	61	93	75
4687	Ben Beardless H	2	127	46	47	46
4726	.....	1	17	.....	.....	65
4801	 2	2	129	5	15	10
4821	Dorsett Bk	6	358	0	2	1
4822	..... P, N	2	109	22	44	33
4823	..... Bk	6	337	0	2	1
4827	.....	2	148	35	37	36
4893	.....	2	86	47	85	65
4924	.....	3	142	2	5	3
4951	.....	2	85	25	43	32
5027	Spartan 2, S	3	182	0	0	0
5028	Wis. Ped. 37 S	2	27	13	25	18
5030	Regal	4	196	10	32	14
5072	Mensury	2	57	37	96	60
5105	Wisconsin Barbless (Ped. 38) S	11	737	0	25	14
5220	Harumaki	2	91	5	67	40
5221	Makishu	2	93	0	8	5
5267	Peatland	9	459	24	59	38
5272	Comp. Cross Sel.	2	126	2	9	6
5274	Comp. Cross Sel.	2	76	25	44	32
5346	do.	6	256	10	65	34
5409	do. 2	2	84	18	18	18
5419	do. D	2	42	5	25	15
5459	do. D	2	107	19	21	20
5673	Dryland S	1	39	.....	.....	62
5757	.....	2	91	2	4	3
5788	.....	1	29	.....	.....	69
5817	..... P	1	18	.....	.....	72
5827	..... P	2	46	30	47	37
5899	Murasaki Mochi P, N	2	79	0	0	0
5979	Colsess IV H	9	180	27	86	68
6001	S.D. 1340 S	2	51	29	33	31
6036	Pliter S	2	70	35	50	44
6051	Missouri Early Beardless H	2	57	13	96	68

TABLE 1.—(*Continued*)

C.I. No.	Name and description[a]	Tests	Total plants	Stripe infected plants		
				Low	High	Weighted average
		No.	*No.*	*Per cent*	*Per cent*	*Per cent*
6087	Sanalta 2, SS	2	64	93	94	94
6088	Newal S	7	354	5	30	18
6093	Brandon 1099 S	4	214	17	50	36
6109	Velvon S	3	164	11	24	16
6112	Stewart	2	84	0	0	0
6239	Ioglos S	4	235	17	77	40
6251	Olli	7	199	21	42	37
6352	Cebada 97A	2	90	4	7	5
6492	Manchurian (Wis. 122–3)	4	283	48	79	68
6503	Tystofte Kors	4	169	0	7	3
6531	Persicum 064 (K) 2, Bk, S	3	182	0	0	0
6533	Viner 1163 2	2	131	8	19	14
6544	Abed Juli	2	63	6	36	27
6557	Solenbyg	1	16	.....	.....	63
6572	Brachytic (Minn. 78–4) N	2	93	0	2	1
6614	.....	2	74	86	87	87
6969	Kindred	2	53	79	88	83
6991	Warrior H	4	175	0	8	4
7011	Minn. II–31–19 S	2	78	27	33	30
7015	Minn. II–31–45 S	2	68	11	18	15
7030	Plush S	5	216	23	95	51
7055	Titan 2	2	110	8	13	10
7069	X191–2–1–2 S	6	646	13	37	17
.....	CP170 2	2	143	6	8	7
.....	CP127422 2	4	223	0	6	1
.....	Garton's 3	2	142	76	85	81
.....	HR346	6	350	13	42	31
.....	Michigan 110 2, S	2	94	0	0	0
.....	Trebi sel. (many leaves)	2	101	12	32	21
.....	Olli × Asplund	2	76	13	25	20
.....	S.S. 1446–4	2	54	11	22	17
.....	Tammisto 0432	2	49	25	46	41
.....	WxWx	3	137	65	88	75
.....	Wxwx	3	120	70	92	85
.....	wxwx (waxy)	5	209	79	98	87
.....	Georgine Pedigree 2	2	75	16	42	25
.....	Belgian 2	2	55	13	17	15

blue aleurone. Exceptions to these characteristics are indicated in the column containing the name of the varieties.

DISCUSSION OF RESULTS

An examination of the results in tables 1 and 2 shows that the tests were severe insomuch as a large proportion of varieties have more than 65 per cent stripe. Another measure of the severity of inoculation conditions was shown by the 75 per cent infection of Oderbrucker (C.I. 4666, Wisconsin Pedigree 5–1) which was used as an inoculated check included 91 times in the series of tests. Most of the varieties were given 2 inoculation tests, while some susceptible varieties were given only 1 test, and still others were given as many as 11 tests. The columns carrying the low and high infec-

TABLE 2.—*Stripe reaction of barley hybrid selections when artificially inoculated*

Hybrid selection[a]	Tests	Total plants	Stripe infected plants		
			Low	High	Weighted average
	No.	*No.*	*Per cent*	*Per cent*	*Per cent*
X120–5–26–12–2	7	324	4	67	23
X152–14–2	5	129	0	56	14
X154–2–79	3	168	13	14	14
–14–75	3	114	8	35	25
X156–8–4–4–3–4–1–2	4	193	42	62	55
X173–5–4–1	4	212	39	78	51
–7–1–6	4	170	36	69	51
–10–5–6–1	4	191	52	86	75
X174–10–2–3	3	122	11	58	35
X181–1–2–1	3	119	3	13	6
X181–2–1–2	3	131	2	13	6
X182–2–1	4	132	0	25	17
–8–1–2	3	119	29	39	33
–8–2–8	3	90	40	70	56
–8–3–3	3	152	16	49	46
X182–8–3–4–1	3	71	17	60	45
–8–3–7	4	121	39	74	58
–10–4–4–1	3	76	3	27	12
–16–4	2	84	0	32	20
–21–2–6–1	4	135	10	30	21
X182–28–2	3	128	31	43	39
–43–1	3	74	0	40	18
–79–1–1	4	159	5	25	14
–145–2	5	176	11	33	22
–175–1	3	133	44	69	55

[a] Parentage of hybrid selections are as follows:
X39—Oderbrucker (Wis. Ped. 5) × Lion (Wis. 117)
X60—Lion × Oderbrucker
X102—Oderbrucker (Wis. Ped. 6, C.I. 1529) × X60–1
X105— do. × X39–3–9
X120—X39–6–2–6 × Oderbrucker (Wis. Ped. 6)
X152—X102–2–3–2 × Korsbyg (Wis. 97–3, C.I. 918)
X154—Oderbrucker (Wis. Ped. 5–1, C.I. 4666) × Korsbyg (Wis. 97–3)
X156—X105–2–7–1 × July (Wis. 106, C.I. 4289)
X168—X39–5–8–4–1 × Oderbrucker (Wis. Ped. 6)
X173—X39–9–3–6–8 × Oderbrucker (Wis. Ped. 5–1)
X174—X39–9–3–6–1 × do.
X181—Wis. Barbless (C.I. 5105) × Korsbyg
X182— do. × Olli (C.I. 6251)
X183— do. × C.I. 4561
X184— do. × Peatland (C.I. 5267)
X191— do. × Newal (C.I. 6088)
X202—Newal × Oderbrucker (Wis. Ped. 5–1)
X212—Chevron (C.I. 1111) × X168–5–1
H4—Wis. Barbless × Chevron
H18—X152–14–1 × Chevron
H23—(Wis. Barbless × Chevron) × Wis. Barbless
H25—(Wis. Barbless × Cross, C.I. 2492) × Wis. Barbless
H27—(Velvet, C.I. 4252 × Korsbyg) × Velvet
H35—(X152–14–1 × Chevron) × X152–14–1
H41—Oderbrucker (Wis. Ped. 5–1) × Chevron
H42—Bolivia (C.I. 1257) × Chevron
H47—Chevron × Trebi (C.I. 936)
H80—Chevron × Hillsa (C.I. 1604)

TABLE 2—(*Continued*)

Hybrid selection	Tests	Total plants	Stripe infected plants		
			Low	High	Weighted average
	No.	*No.*	*Per cent*	*Per cent*	*Per cent*
X182-175-2-1	4	116	4	52	20
-202-2	4	136	4	23	12
-243-1	4	141	0	3	1
X183-1	3	103	5	34	29
-5	4	172	5	26	13
X184-4-4	3	138	21	37	34
-5	4	135	6	20	12
-11	2	92	6	9	8
-11-15-1	4	168	6	29	12
X191-2-2-3	5	208	6	37	19
X191-2-3	2	108	5	6	6
-3-2	3	118	2	52	15
-13-1	2	111	15	33	27
-13-2	2	103	20	32	25
-16-2-1	5	170	9	36	26
X191-16-3-1	5	215	12	40	30
-17	3	115	8	67	37
-19-1	3	135	6	37	15
-24	3	132	7	31	18
-25	5	168	20	31	25
X202-1	2	68	15	15	15
X212-1	4	197	3	41	21
H4-3-5-6-2-1-1	4	181	0	18	7
-2	3	146	5	22	10
-3-10-1-1-1	4	129	0	6	2
H4-3-10-4	4	171	10	36	25
-5-1-3-11-4	4	169	4	16	7
-5-1-7-6	5	180	4	15	9
-5-1-12-6-2	2	111	2	11	6
-5-1-12-6-5	3	123	9	16	12
H4-5-6-5-3-2-1	2	99	6	25	18
-5-6-8-3-1-1	4	171	0	13	5
-8-2-2-3-5	2	123	2	6	4
-8-6-1	3	139	6	39	17
-8-7-3	5	270	5	23	14
H4-8-7-3-1-1	4	200	4	36	20
-10-3-6-1-1	4	163	7	24	16
-3-5-6-2-1-3	2	105	5	5	5
H18-3-1-4-1-8	4	151	0	9	4
H23-2-17-4-2	2	147	11	16	13
H25-16-2-1-1	4	176	3	17	12
H27-3-7-4-4	2	124	0	0	0
H35-7-2-1-3	4	138	9	33	20
-7-6-4-1	3	145	27	68	53
H41-4-4-4-1	4	239	8	17	8
H41-4-8-2-1	2	120	8	11	9
H42-5-4-9-9	2	103	61	70	65
-5-4-10-7	2	117	48	53	50
H47-91	2	36	17	22	19
H80-4-2-1	2	72	6	7	7

tions illustrate the variation that was experienced in stripe testing with the method described. Examples: important differences occur between trials with C.I. 241 and C.I. 276, while insignificant differences appear with C.I. 531 and C.I. 652. A lesser number of varieties show identical percentages,

as with C.I. 595. A total of 12 varieties had 90 per cent or more infected plants. They were the following varieties: C.I. 967, C.I. 998, C.I. 1137, C.I. 1315, C.I. 1330, C.I. 1409, C.I. 1417, C.I. 1551, C.I. 2208, C.I. 4500–1, C.I. 4567–1, and C.I. 6087. Stripe symptoms of C.I. 998 were decidedly different from those of the Oderbrucker-Manchuria type. In C.I. 998 striped plants were greatly reduced in size and culm elongation. In contrast striped plants of the Oderbrucker type were only slightly reduced in height. In response to infection, plants of some varieties were distinctly stunted and rosetted, and with little tendency to produce typical long striped lesions on the leaves.

More important from a plant-breeding standpoint, however, are those varieties that resisted the disease. The authors consider that an infection

TABLE 3.—*Source and number of varieties with less than 15 per cent stripe infection or more than 45 per cent*

Source or country	Number of varieties with	
	Less than 15%	More than 45%
Abyssinia	4	8
Afghanistan	1	.....
Asia	2	.....
Asia Minor	1	.....
Australia	2	.....
China	6	1
Chosen	1	.....
Denmark	1	.....
Europe	2	.....
India	8	1
Italy	2	.....
Japan	4	1
Manchuria	6	14
Mesopotamia	1	.....
Russia	3	4
Siberia	1	2
South Africa	1	.....
Sweden	4	1
Switzerland	2	.....
Total	52	32

of 15 per cent or less indicates that a variety is resistant. Thirty-one varieties showed no infection, and 81 more developed less than 15 per cent stripe, making a total of about 112 with resistance. A classification of stripe reaction might be defined as follows: 0–1 per cent = highly resistant; 2–15 per cent = resistant; 16–30 per cent = moderately resistant; 31–45 per cent = intermediate; 46–60 per cent = moderately susceptible; 61–100 per cent = susceptible.

Of the 284 varieties in the C.I. collection tested for stripe reaction and whose eastern hemisphere source is known, 52 varieties had less than 15 per cent stripe and 32 had more than 45 per cent. For convenience, the number and source of these varieties falling into the two classes are given in table 3. Resistant varieties came from different locations of Europe, Asia, and Africa. Manchuria and Abyssinia provided more susceptible types than other countries.

Of the varieties grown commercially in the north-central States Spartan is highly resistant. Hannchen, Regal, Trebi and Wisconsin Barbless are resistant, while Glabron, Newal, Odessa, and Velvon are moderately resistant. Ioglos, Olli, Peatland, Pliter, and Velvet are intermediate, while Dryland, Kindred, the Manchurias, Missouri Early Beardless, and Oderbrucker are susceptible to stripe. Summarized in table 4 are the percentages of stripe obtained in these varieties.

The hybrid selections listed near the end of table 2 vary considerably in their response to stripe, some being resistant, some susceptible, and others

TABLE 4.—*Stripe reaction of some commercial barley varieties of the United States and Canada*

Variety	C.I. number	Average stripe infection
		Per cent
Dryland	5673	62
Glabron	4577	30
Hannchen	531	7
Ioglos	6239	40
Kindred	6969	83
Manchuria, N. D. 2121	2947	86
Manchuria, Minn. 184	2330	78
Manchuria, O.A.C. 21	1470	83
Missouri Early Beardless	6051	68
Newal	6088	18
Oderbrucker, Wis. Ped. 5–1	4666	75
Odessa	934	23
Olli	6251	37
Peatland	5267	38
Pliter	6036	44
Regal	5030	14
Spartan	5027	0
Trebi	936	5
Velvet	4252	45
Velvon	6109	16
Wisconsin Barbless	5105	14

intermediate. Most of the selections are smooth-awned, though a few have rough awns; some have good malting quality; and some have resistance to other important diseases. Most of the smooth-awned selections are deficient in one or more desirable characteristics, and, therefore, would not be considered for replacement of varieties now used for commercial production.

A few of the stripe-resistant varieties that are of interest from the standpoint of their use as breeding stocks are as follows: C.I. 918, C.I. 920, C.I. 936, C.I. 1111, C.I. 1613, C.I. 1969, C.I. 2276, C.I. 2492, C.I. 4821, C.I. 5105, and C.I. 6352. Some of these are particularly valuable because of their resistance to other important diseases, as well as to stripe. Shands (10) has pointed out the value of Chevron (C.I. 1111) as a breeding stock, since it carries resistance to stem rust, mildew, scab, and stripe.

SUMMARY

Over a period of 8 years 375 barley varieties and selections were tested for their reaction to the stripe disease by bringing germinating barley kernels into contact with the mycelium of *Helminthosporium gramineum* Rabh.

Some varieties were highly resistant to the cultures of the fungus used in these tests; other varieties were intermediate in their stripe reaction; while still others were susceptible. Oderbrucker (C.I. 4666), which was used as the inoculated check 91 times in the series of tests, averaged 75 per cent stripe-infected plants. This indicates that the method of inoculation was effective. Physiologic specialization of the fungus may influence varietal response to inoculation. The host response to stripe infection may differ distinctly between varieties.

Some of the stripe-resistant varieties may be of value as breeding stocks because they have other desirable characters.

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INHERITANCE OF CHLAMYDOSPORE AND SORUS CHARACTERS IN SPECIES AND RACE HYBRIDS OF TILLETIA CARIES AND T. FOETIDA[1]

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The demonstration of hybridization between *Tilletia caries* (DC.) Tul. (*T. tritici* (Bjerk.) Wint.) and *T. foetida* (Wallr.) Liro (*T. levis* Kuehn) by Flor (2) suggested the possibility of studying the inheritance of certain physiological and morphological properties of these organisms, such as pathogenicity, chlamydospore markings, and spore ball characters. That the expression of these characters is genetically controlled is shown in the results of experiments reported by several investigators, although the manner of their inheritance has not been determined. For example, Flor (2) observed that species-hybrid spores were smooth, like the *T. foetida* parent, and Hanna (4) made similar observations. However, neither of these workers observed segregation into smooth and reticulate spores in the progeny of the hybrid spores. Furthermore, Hanna (4) states that the spore balls of the hybrids resembled the *T. foetida* parent spore balls and that the characteristic trimethylamine odor of the *T. foetida* parent was present in the hybrids. Apparently, however, no segregation for these characteristics was observed in any of the hybrids. Likewise, Becker (1) found culture characters to be of a heritable nature but the manner in which they are inherited was not determined, although the inheritance of pathogenicity was found to be intermediate in one cross and recessive in another.

In 1938 (7) the writer described a hybrid-race of *Tilletia caries,* derived from a cross between T–9 (*T. caries*) and L–8 (*T. foetida*). The F_1 spores of this hybrid were reticulate, although the reticulations were less prominent than those of the *T. caries* parent. In this hybrid, therefore, the reticulate character was at least partially dominant. No segregation of spore characters was observed. Later, however, studies (8) with hybrids produced by crossing the dwarf bunt with L–8 and T–12 revealed that the hybrid spores were intermediate between the parent types in the F_1 and that segregation occurred in the F_2. This was the first published evidence of segregation of factors for chlamydospore characteristics in hybrids of the bunt fungi. More recently (10), a report on the inheritance of pathogenicity was published in which it was shown that factors for pathogenicity and chlamydospore characters are inherited independently. The purpose of

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this report is to present some detailed observations on the inheritance of factors governing chlamydospore and spore-ball characters in certain hybrids between *T. caries* and *T. foetida.*

MATERIAL AND METHODS

Fifty-five hybrids were used in these studies, of which 32 were hybrids between *Tilletia caries* and *T. foetida* and 23 were hybrids between races within these species. The races used were, T–8, T–9, T–10, T–12, L–7, L–8 (9), and dwarf bunt (8). Hybrids were produced by the method already described (6), each one being designated by number, and pedigreed as to race, chlamydospore, and sporidium.

Chlamydospores of the F_1 generation were obtained from Hindi (C.I. 8454) wheat, which had been inoculated with paired monosporidial lines. Attempts to culture complete sets of individual sporidia from F_1 spores

TABLE 1.—*Summary of observations on chlamydospore characters of hybrids between Tilletia caries and T. foetida and their respective races*

Races crossed	Number of hybrids	Number of hybrids with F_1 spores		Remarks[a]
		Smooth	Reticulate	
L– 8 × T– 9	6	3	3	Reticulations small.
L– 7 × T–10	8	4	4	Reticulations medium to large.
L– 8 × T– 8	1	1	0	Spores uniformly spherical (like T–8 parent).
L– 8 × T–12	7	0	7	Reticulations small.
T– 8 × T– 9	9	0	9	Reticulations large and variable in shape.
L– 8 × L –7	2	2	0	Spores irregular in shape.
T– 8 × T–10	4	0	4	Reticulations medium to large.
T–12 × Dwarf ...	8	0	8	Reticulations medium to large.
L– 8 × Dwarf ...	10	0	10	Reticulations medium to large.
Total	55	10	45	

[a] The reticulations on the chlamydospores of all of the *T. caries* parents were large.

were unsuccessful and for this reason chlamydospores were used for inoculum to produce the F_2 generation on the susceptible winter wheat variety Hybrid 128 (C.I. 4512). Obviously, chlamydospore material obtained thusly was not readily adapted to an exact factorial analysis. Nevertheless, it was suitable for a study to determine the nature and extent of variation in chlamydospore characters of hybrids between species and races. Microscopic observations were made, therefore, to determine the dominant or recessive nature of certain chlamydospore characters in the F_1 and the nature and extent of segregation of these characters in the F_2.

Spore balls of certain races of the bunt fungi differ primarily in size and shape. The expression of these characters, however, usually is governed to some extent by the variety infected. Furthermore, considerable variation within a race may be expected, owing to the difference in the location of the bunt balls on the spike. Consequently, in studying spore-ball characters of parent races and hybrids between them, the spore balls were

selected from the same variety and the same relative position on the spikes, usually the central portion.

EXPERIMENTAL RESULTS

Chlamydospore Characters

The observations on chlamydospore characters in the F_1 generation are summarized in table 1. Twenty-four of the interspecies hybrids had reticu-

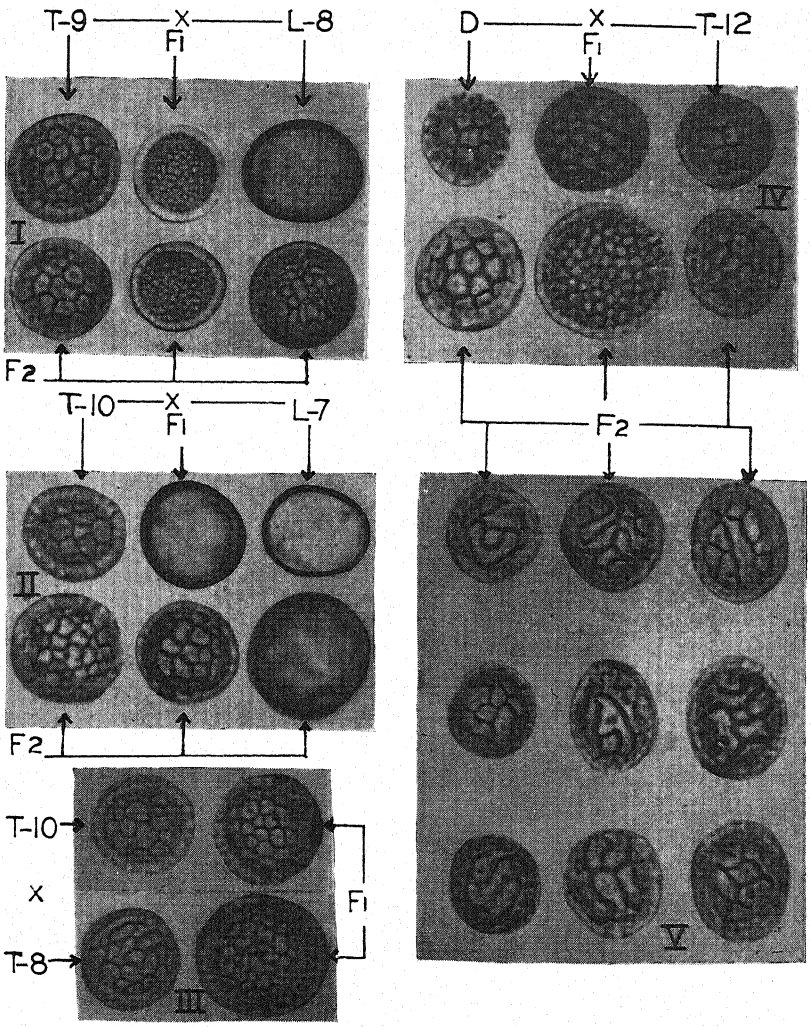


FIG. 1. Photomicrographs of parent and hybrid chlamydospores of *Tilletia caries* and *T. foetida* showing heritable nature of spore markings. (Enlarged and retouched). I. Races T–9 × L–8. II. Races T–10 × L–7. III. Races T–10 × T–8. IV. Dwarf bunt × T–12. V. Cerebriform F_2 segregate from F_1 of IV. × about 850.

late spores and 8 had smooth spores. The spores of hybrids between races of *T. foetida* were smooth and those of hybrids between races of *T. caries*

were reticulate. As indicated in table 1 and illustrated in figure 1, the reticulations of the hybrid spores were highly variable in character. For example, in hybrids between L–8 and T–9 the reticulations were extremely small (Fig. 1, I) and the reticulations of the spores of hybrids between L–8 and T–12 were similar in character. On the other hand the reticulations of the spores of several other species hybrids ranged in size from medium in some to large in others. This suggests lack of complete dominance of the reticulate character in any of the interspecies hybrids studied. Furthermore, in hybrids between races of *T. caries,* there was lack of complete dominance of reticulation type represented by different races. This is indicated by the wide variation in size and shape of reticulations on F_1 chlamydospores of hybrids between T–8 and T–10 (Fig. 1, III) and the reticulations of F_1 chlamydospores of hybrids between the dwarf bunt and T–12, which were distinctly different from those of either parent (Fig. 1, IV). As already stated, 8 of the interspecies hybrids had smooth F_1 spores, indicating complete dominance in these hybrids of the smooth character of the *T. foetida* parent (Fig. 1, II).

Although there was considerable variability in chlamydospore markings in the F_1 generation of some of the hybrids, the nature and degree of variability in the F_2 was clearly indicative of segregation of factors that control these characters. For example, in certain hybrids between T–9 and L–8 some of the F_2 spores resembled the T–9 parent, some resembled the F_1 spores, and others had reticulations that were highly variable in character (Fig. 1, I). The L–8 parent type (smooth spores) was not recovered in the F_2. On the other hand, in hybrids between T–10 and L–7 which had smooth F_1 spores, both parent types in addition to other types were recovered in the F_2 (Fig. 1, II). Similar results were obtained with hybrids between the dwarf bunt (D) and T–12 (Fig. 1, IV). Worthy of special note is the cerebriform type illustrated in figure 1, V. Although not present in all sori, this type of spore was predominant in some of them. They have not been found to be viable.

Sorus Characters

That sorus characters are genetically controlled, at least to some extent, is indicated by the results of a study with Hybrid 39, which resulted from a cross between T–9 and L–8. The spore ball types of the parents and hybrids are illustrated in figure 2. It will be noted that there is only a slight difference in the sori of T–9 and L–8 on the variety Hybrid 128 while the sori produced by Hybrid 39 (F_6) on this variety are much smaller than those of these two races. Furthermore, the hybrid sori are smaller on Hohenheimer than the T–9 parent sori and smaller on Oro than the L–8 parent sori. Thus it appears evident that certain factors governing the size of the sori were operative in this hybrid between T–9 and L–8, which resulted in the production of sori smaller than those of either parent. Furthermore, the fact that the hybrid sori were smaller than the parent

sori suggests transgressive inheritance. Also there appears to be a slight difference in the shape of the sori of the hybrid and L–8 on Oro (Fig. 2). This is regarded as some evidence that sorus shape likewise is genetically controlled. Similar observations have been made in connection with other hybrids.

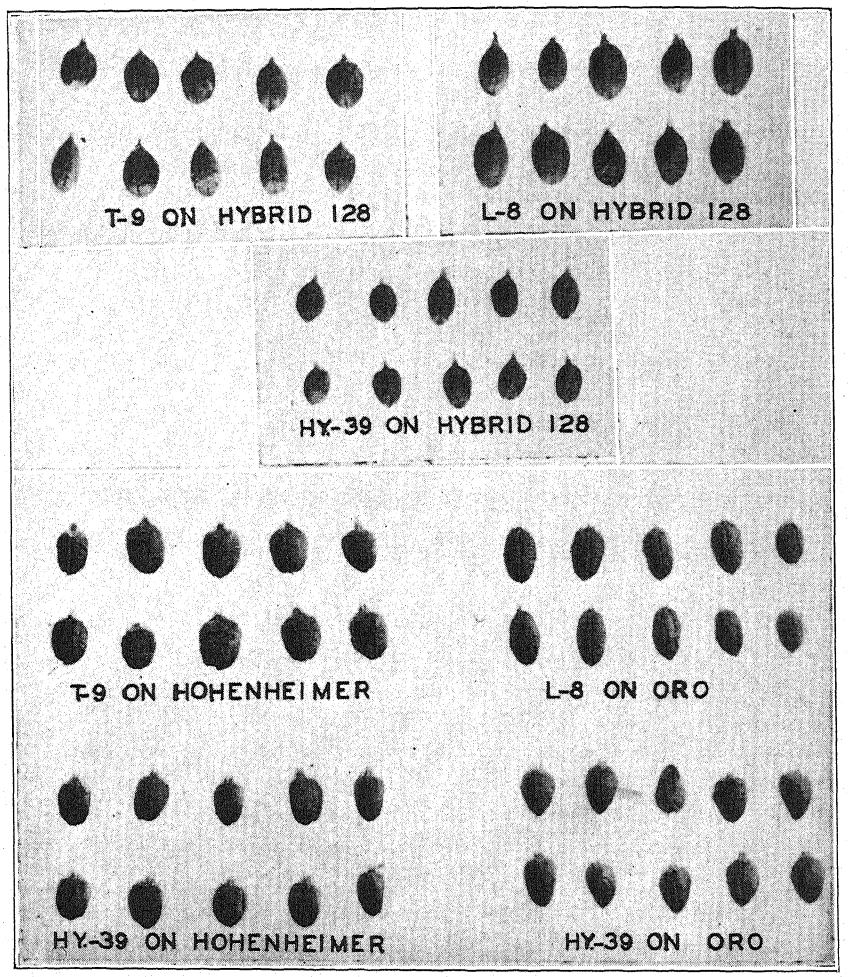


FIG. 2. Type of sorus produced on 3 varieties of wheat by races T–9 and L–8 and a hybrid between these races.

DISCUSSION

The exact nature of inheritance of factors governing chlamydospore and sorus characters could not be determined in this study, mainly because complete culture sets of primary sporidia from individual chlamydospores could not be obtained. Nevertheless, there is conclusive evidence in the data and observations reported that chlamydospore markings and size of

the sorus are determined by genetic factors and that wide variations in these characters probably are the result of different combinations of genetic factors. Consequently, one might expect to encounter variable types in collections of bunt from commercial wheat fields, inasmuch as hybridization might occur under natural conditions. This is especially true with regard to the reticulations of the chlamydospores of *Tilletia caries,* which, in the hybrids studied, were found to range in size from extremely small to relatively large.

That hybrid spores have been observed in field collections is indicated by the reports of several investigators in different parts of the world. In the United States, Flor (2) found *Tilletia caries* and *T. foetida* occurring together in field collections in the majority of cases, suggesting ample opportunity for hybridization. Furthermore, he observed all degrees of reticulation on spores in field collections, some spores being so finely reticulated as to be difficult to distinguish from the smooth spores of *T. foetida* while others were so coarsely reticulated as to appear spiny. Gassner (3) observed spores that had much finer reticulations than *T. caries* and thin spore walls like *T. foetida.* He regarded this as an intermediate type that resulted from hybridization, and designated it as *T. caries intermedia.* It occurred infrequently (only 8 heads in over 2000 were found) and usually in the presence of the 2 main species, in several cases all 3 being in the same head. This association seems to support Gassner's theory that the intermediate type is the result of interspecific hybridization. Intergrading types, such as those described by Flor, apparently were not observed by Gassner (3). Hirschhorn (5), however, describes and illustrates several types of reticulations, intergrading from the typical *T. caries* type to Gassner's *intermedia* type. She suggested that these intermediate types resulted from hybridization between *T. caries* and *T. foetida.* This suggestion is logical, since the experimental evidence now at hand indicates that the atypical spores observed by Flor (2), Gassner (3), and Hirschhorn (5) did result from hybridization between the two species. Furthermore, from the taxonomic viewpoint, the importance of recognizing this possibility is emphasized by the fact that Savulescu *et al.* (11) established the species *T. triticoides* as distinct from *T. caries* on the basis of its slightly smaller spore size and more delicate reticulations. In other words, because of the intergrading types of chlamydospores that result from hybridization, delimitation of species of *Tilletia* on wheat according to size of reticulations can scarcely be justified.

SUMMARY

In some hybrids between *Tilletia caries* and *T. foetida* the smooth character of the *T. foetida* spores was completely dominant over the reticulate character of the *T. caries* spores. In other hybrids the reticulate character was partly dominant over the smooth character. Variability in type of reticulations and size of spores was observed in the F_1 generation of certain hybrids between races of *T. caries.*

Segregation and recombination of factors for chlamydospore characters occurred in the F_2 generation of hybrids between *Tilletia caries* and *T. foetida* and between races within species. Spores resembling both parents and spores distinctly different from both parents usually were recovered in the F_2. In one species hybrid smooth-type spores of the *T. levis* parent were not recovered in the F_2.

Evidence is presented indicating that size and shape of the sori of *Tilletia caries* and *T. foetida* are, at least partly, genetically controlled.

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PRELIMINARY REPORT ON SOME MOSAIC DISEASES OF IRIDACEOUS PLANTS

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Comparatively few members of the Iridaceae are reported subject to mosaic diseases, and none of these plants are recorded as suscepts of the well-known viruses of wide host range. Iris mosaic (2) has been shown to be transmissible by sap, and by the aphids *Macrosiphum solanifolii* (Ashm.) and *Myzus persicae* (Sulz.), but the known host range is limited to the genus *Iris*. A mosaic disease of gladiolus, described by Dosdall in 1928 (4), has assumed greater importance in recent years (3). Although shown to persist in vegetative parts, gladiolus mosaic has not previously been shown to be transmissible. A virus disease of Freesia, not known to occur in the United States, has been described briefly and attributed to Freesia virus 1 by Smith (6), but no report of transmissibility has yet appeared. Atanasoff (1) and later observers have reported mosaic symptoms in Crocus.

Corms of Tigridia (*T. pavonia* (L.f.) Ker) were received from a grower in western Washington in 1939, with the report that a mosaic-like disease had spread rapidly through a previously normal planting. Mosaic symptoms are common in gladiolus varieties grown at the Plant Industry Station, Beltsville, Maryland, and elsewhere. Similar mottling symptoms have been noted in plants received from commercial sources, including *Babiana* hybrids, *Ixia* hybrids, *Sparaxis* hybrids, *Streptanthera cuprea* Sweet, *Tritonia lineata* (Salisb.) Ker, *Tritonia* hybrids, including *Montbretias*, and *Watsonia marginata* (L.f.) Ker. Stocks from New Jersey, Ohio, and California dealers appear similarly affected, indicating that the virus or viruses are generally distributed in the United States. A study of the interrelationship of these virus diseases, and of their possible relationship to such diseases of other monocotyledons, was begun in 1940. Although the work is far from complete, transmissibility of mosaic viruses in *Tigridia*, *Gladiolus*, and several other genera may be reported at this time.

SYMPTOMS

Symptoms of the diseases in the several source plants had the following characteristics:

Tigridia mosaic, appearing in the greenhouse in plants grown from corms received from the Washington grower, consisted of pale-green to yellowish-green, irregular streaks and blotches (Fig. 1, A) in leaves and flower bracts. Breaking of the flower color in the form of pale, white streaks or, less com-

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monly, dark streaks, has been detected in *Tigridia,* but these symptoms have been less striking than the breaks commonly present in the flowers of mosaic-affected bulbous iris.

A number of gladiolus plants of the variety Picardy were selected for flower breaks in the field in 1941 and grown under glass in 1942. Young leaves developing in April and May showed a fine-grained, angular, green mottling, and the flowers showed distinct breaking in color. In this sample

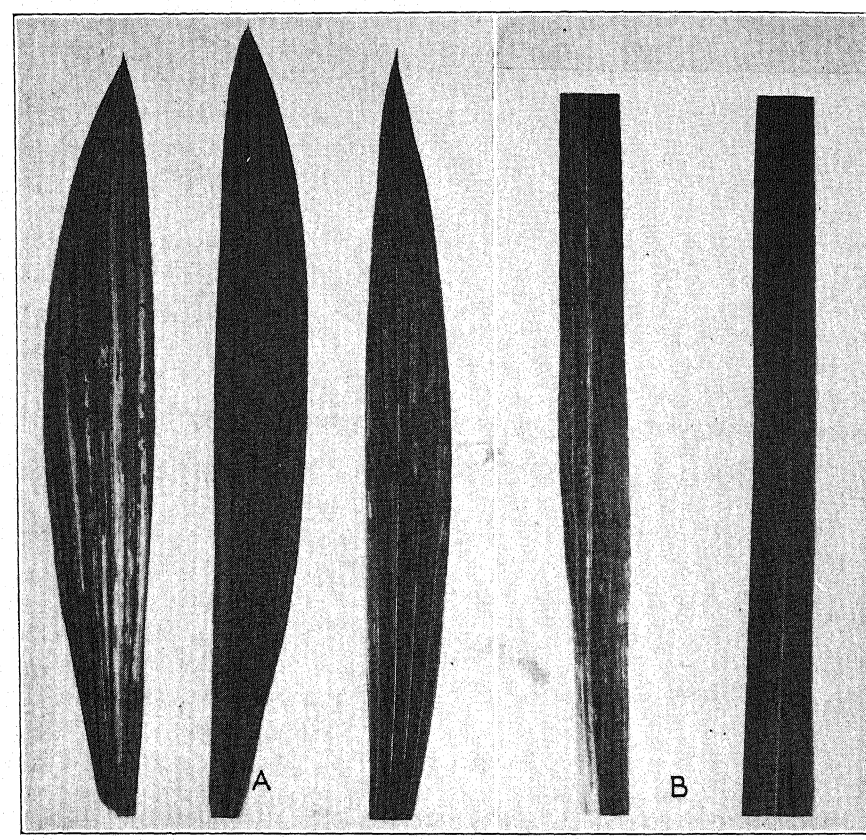


FIG. 1. A. Tigridia mosaic. Left to right, natural infection, control, and experimentally produced infection (*Aphis gossypii* transfer). B. Gladiolus mosaic. Experimentally produced (*Myzus persicae* transfer) and control.

of Picardy the flowers were pink in bud, but became pale-streaked or spotted when full-blown or while fading. Both color-intensified and bleach patterns were noted in other lots of this variety and in other varieties. The corm symptoms noted by Dosdall (4) have not been detected in our material.

Characteristic mosaic mottling appeared in plants of the other genera mentioned above. A bright-yellow, angular mottle was present in naturally infected *Ixia* hybrids, light and dark green mottling in the others. Darker "tear-drop" flower breaks were noted in *Babiana* hybrids, and pink streak

breaks were present in white flowers of affected *Ixias*. *Sparaxis* hybrids exhibited a strong green leaf mottling, with frequent crinkling and pinching of affected leaves not observed thus far in other iridaceous plants.

TRANSMISSIBILITY

The carborundum leaf-rubbing technique failed to transmit Tigridia-mosaic virus to Tigridia seedlings, or gladiolus-mosaic virus to gladiolus seedlings. The methods of culturing and transferring aphids were those described in connection with other studies (5). Control seedlings of *Tigridia, Gladiolus,* and *Tritonia crocata* (L.) Ker have remained healthy in the screened and fumigated greenhouse throughout this study.

The data in table 1 show that Tigridia-mosaic virus is transmissible by *Aphis gossypii* Glover. Transfers by *Macrosiphum lilii* (Monell) and by *Myzus circumflexus* (Buckt.), indicated by single infections by each species, also are considered authentic, as no evidence of contamination was detected in the greenhouse-grown seedlings under test. Mosaic mottling typical of the source material appeared in experimentally infected plants, but no flower breaks were observed.

Gladiolus-mosaic virus was transmitted to seedling gladiolus plants by *Myzus persicae* in three trials (Fig. 1, B) and by *M. circumflexus* in one trial. Symptoms appeared in 2 to 4 weeks in actively growing plants, or else in the second season. Flower breaks like those in the Picardy source appeared in the 6 experimentally infected plants that bloomed. Uninoculated seedlings developed no breaks like the virus type, but some of these produced flowers with fine striping or marbling believed to be heritable color patterns.

Tritonia crocata seedlings offered some promise as test plants for iridaceous viruses. This species is readily grown from seed under glass, but is not ideal in symptom expression. Some exploratory transfers to this species are listed in table 2. *Myzus persicae* transmitted virus from mosaic-affected *Babiana, Ixia, Sparaxis, Streptanthera, Tigridia, Tritonia,* and *Watsonia* to *Tritonia crocata* seedlings. Mottling appeared at the base of young leaves 2 to 8 weeks after exposure, as fine, angular, dark-green islands on a paler green ground. No flower breaks were detected in *Tritonia.*

No certain distinctions between viruses transferred from different genera can be made from these data (Tables 1 and 2). Apparent differences noted in vector relations, ease of transfer, or incubation periods may well be due to differences in growth rate of inoculated seedlings or to other factors. In other tests, however, some differences in symptom expression indicate that distinct viruses or virus strains are concerned in iris mosaic from German iris and in the mosaic of *Sparaxis*. *Myzus persicae* transferred from mosaic-affected German iris to gladiolus seedlings produced a prominent rectangular mottle pattern in 2 weeks, the 2 affected shoots, of 5 exposed, later turning yellow, then brown, and dying prematurely. When this test was repeated with the same vector, small side shoots were mottled and killed in

a similar sequence in 4 pots of 5 exposed, but larger plants in the same pots remained unaffected. Apparently a virus from mosaic-affected German iris is lethal to gladiolus, whereas the gladiolus-mosaic virus from Picardy

TABLE 1.—*Transmission of virus from mosaic-affected Tigridia to Tigridia seedlings, and from mosaic-affected gladiolus to gladiolus seedlings, by aphids*

Date of transfer	Aphid species	Aphids per plant	Seedling plants infected[a]	Minimum incubation period[b]
	Tigridia mosaic			
		Number		*Days*
May 29, 1941	*Aphis gossypii*	25	3/9	20
June 19, 1941	do.	25	2/5	21
June 1, 1942	do.	20	3/6	26
June 21, 1941	*Aphis rumicis* L.	20	0/10	.....
May 28, 1942	do.	25	0/5	.....
May 29, 1941	*Macrosiphum lilii*	20	1/8	340
June 23, 1941	*Myzus circumflexus*	20	1/3	17
	Gladiolus mosaic			
July 30, 1942	*Macrosiphum solanifolii*	30	0/10	.....
Aug. 26, 1942	do.	10	0/4	.....
May 28, 1942	*Myzus circumflexus*	25	2/5	252
May 28, 1942	*Myzus persicae*	25	4/5	25
June 26, 1942	do.	25	4/6	221
Feb. 5, 1943	do.	40	5/5	14

[a] Number infected over number exposed.
[b] Days until symptoms appeared.

TABLE 2.—*Transmission of virus from mosaic plants of several iridaceous genera to Tritonia crocata seedlings by Myzus persicae*

Date	Source of virus	Aphids per test	Plants infected[a]	Minimum incubation period[b]
		Number		*Days*
Dec. 31, 1942	Babiana hybrid	80	7/16	14
Feb. 5, 1943	Gladiolus (var. Picardy)	240	0/20	.....
Feb. 5, 1943	*Iris germanica* L.	240	0/20	.....
Dec. 31, 1942	Ixia hybrid	80	2/20	14
Jan. 30, 1942	Sparaxis hybrid	250	1/10	56
Jan. 2, 1943	do.	80	2/20	48
Feb. 5, 1943	do.	240	0/20	.....
Dec. 31, 1942	*Streptanthera cuprea*	80	11/15	14
May 28, 1942	*Tigridia pavonia*	150	1/5	19
Feb. 5, 1943	do.	240	0/20	.....
Dec. 31, 1942	Tritonia hybrid	80	4/18	14
Dec. 31, 1942	*Watsonia marginata*	80	3/20	50

[a] Number of plants infected over number exposed.
[b] Days until symptoms appeared.

induces mild mottling and flower breaks only when transferred by the same vector.

Transfers of *Myzus persicae* from mottled *Sparaxis* produced positive results but no unusual patterns in *Tritonia crocata*. A transfer to gladiolus

seedlings yielded mosaic mottling in 3 of 5 exposed, 1 of which developed a prominent, rectangular, yellow pattern similar to the yellow mottling occasionally seen in gladiolus in the field. Transfers by *M. persicae* from *Sparaxis* to Clara Butt tulip repeatedly induced white spotting in leaves and cucumber-mosaic-type flower breaks. Mechanical transfers to tobacco from such inoculated tulips, and also from the *Sparaxis* source, yielded a cucumber-mosaic virus strain similar to those commonly present in Easter lilies affected with necrotic fleck. No cucumber-mosaic virus was recovered by mechanical methods from the gladiolus showing exceptional symptoms following inoculation from *Sparaxis*.

In further mechanical transfers cucumber-mosaic virus isolated from *Sparaxis* produced white-ring and watermark primary lesions in Turkish tobacco, followed by systemic mild yellow mottling. In White Spine cucumber, primary lesions appeared in the cotyledons, as was the case with the celery strain, followed by mild yellow mottling but no stem necrosis. *Zinnia elegans* Jacq. developed systemic mottling. White-spot primary lesions of the cucumber-mosaic-virus type were produced in *Mesembryanthemum crystallinum* L.

DISCUSSION

The occurrence of cucumber-mosaic virus in *Sparaxis* is believed to be a new record for the family Iridaceae, as well as for this genus. No evidence has been obtained thus far that other genera of Iridaceae are susceptible. The role of this virus in the mosaic disease, observed to occur naturally in *Sparaxis*, cannot be evaluated until healthy individuals are available for inoculation. The evidence at hand suggests that this virus may contribute to the effects observed but that it is not the only virus concerned in the mosaic disease of *Sparaxis*.

It is uncertain whether gladiolus mosaic, Tigridia mosaic, and the similar diseases observed in several other iridaceous genera are caused by a common virus. The differential response of gladiolus seedlings indicates that iris-mosaic virus, as found in German iris, is distinct. Until these relations are more definitely established, it is considered unwise to propose technical names for the viruses concerned. Common names such as gladiolus-mosaic virus and Tigridia-mosaic virus should be adequate at present.

SUMMARY

Tigridia-mosaic virus was found to be transmissible by *Aphis gossypii, Macrosiphum lilii,* and *Myzus circumflexus,* but not by sap; gladiolus-mosaic virus was transmissible by *M. circumflexus* and *M. persicae,* but not by sap.

Virus was transmitted from mottled *Babiana, Ixia, Sparaxis, Streptanthera, Tigridia,* and *Watsonia* to *Tritonia crocata* by *Myzus persicae.*

Cucumber-mosaic virus was found naturally occurring in *Sparaxis* hybrids.

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RHIZOCTONIA LEAF SPOT OF COTTON

D. C. Neal

(Accepted for publication January 20, 1944)

A leaf spot of cotton, apparently due to the Rhizoctonia fungus, was reported from Louisiana in July, 1943 (3). The disease was first observed by H. B. Brown, Agronomist of the Experiment Station, on leaves of an unknown strain of Deltapine cotton plants growing in a bottom field near a drainage ditch. Subsequently, specimens of the affected leaves were collected and referred to the writer for examination for a possible disease-producing organism. Microscopic study and pure-culture isolations from affected leaves have shown that the Rhizoctonia fungus is responsible for this leaf spot. Also, cultural characteristics and growth relationships indicate that it is in all probability the common soil species, *Rhizoctonia solani* Kühn (*Corticium solani* (Prill. & Del.) Bourd. & Galz.). The disease also was later observed on other varieties, including Coker and Delfos cotton. While frequently a considerable area of the leaf is attacked, causing some shedding, observations made so far show that damage to the plant is not serious enough to cause much reduction in yield.

DESCRIPTION OF THE DISEASE[1]

The disease is readily recognized. In the early stage, light brown, irregularly shaped spots of varying size appear between the veins, bordered by a dark-purplish ring—a distinctive feature. As the fungus advances, it causes the tissues immediately surrounding the spots to become chlorotic, and the necrotic area of the old spot cracks or falls out, presenting a ragged, shot-hole appearance (Fig. 1). Microscopic and macroscopic examinations reveal abundant light- to yellowish-brown mycelium over and around the spots on the lower surface of the leaves. The mycelium agrees closely with morphological characteristics described by Duggar (2) for *Rhizoctonia solani*. Hyphal strands are frequently numerous and may easily be observed with a hand lens.

CULTURAL STUDIES AND GROWTH ON MEDIA

The fungus was easily isolated by sterilizing for brief intervals (2 to 3 minutes) small sections of infected leaves in a 3.5 per cent B–K stock solution (active ingredient calcium hypochlorite) diluted with 2 parts water and subsequently plating on potato-dextrose agar. By using newly affected leaves, especially the chlorotic tissue surrounding the spots, the fungus may be obtained in practically 100 per cent of the platings by this technique. In addition to potato-dextrose agar, the fungus also grows well on Trommer's malt agar, the hyphal strands becoming cinnamon-brown or darker after 6 or 7 days' growth in Petri plates and forming pseudo-sclerotial wefts.

[1] The writer is indebted to Dr. E. C. Tims of the Department of Plant Pathology, Louisiana Agricultural Experiment Station, for the photographs of this leaf spot.

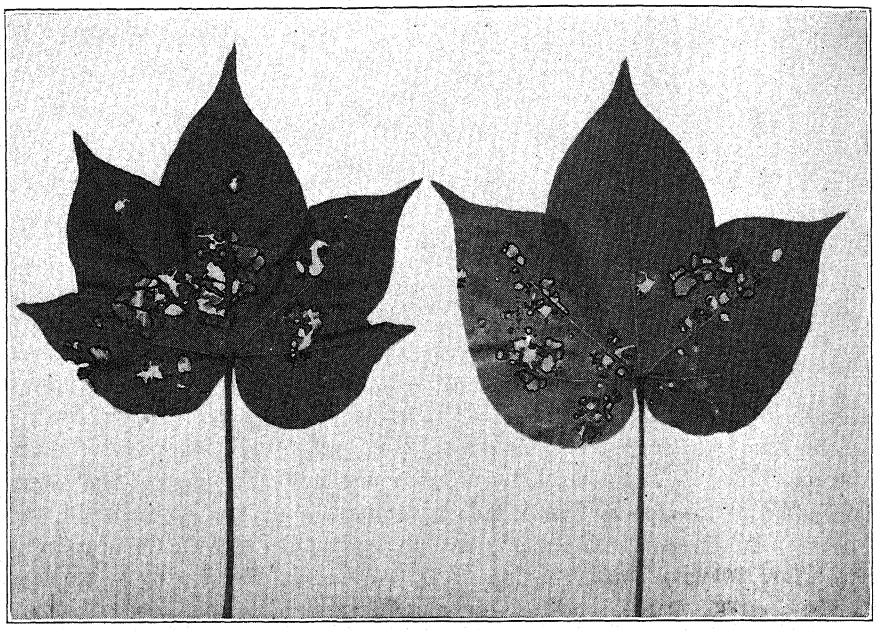


FIG. 1. Cotton leaves infected with *Rhizoctonia* fungus. Note shot-hole appearance of leaves and dark-purplish ring surrounding spots. Natural infection. Variety—Deltapine. Photographed July 16.

PATHOGENICITY TESTS

Typical lesions of the disease have been produced on leaves of plants by inoculating with agar cultures, and the fungus later reisolated from the inoculated leaves. In most cases 3- or 4-day-old cultures of the fungus on potato-dextrose agar in Petri plates were employed for the inoculations, a block of the agar culture approximately a half inch square being applied with scalpel to the upper and lower surfaces of separate leaves. As controls, other leaves were treated similarly with sterile agar blocks. The results of these inoculations are shown in table 1 and illustrated in figure 2.

TABLE 1.—*Number and per cent of cotton leaves with lesions 10 days following inoculation with Rhizoctonia isolate*[a]

Variety	Leaves inoculated			Leaves with lesions			Per cent[b] infection
	Surface placement of inoculations			Surface placement of inoculations			
	Upper	Lower	Total	Upper	Lower	Total	
Coker	10	10	20	6	10	16	80.0
Deltapine	10	10	20	4	8	12	60.0
Delfos	6	6	12	4	6	10	83.3
Coker control	.....	.....	0	.....	.....	0	0.0
Deltapine control	.....	.....	0	.....	.....	0	0.0
Delfos control	.....	.....	0	.....	.....	0	0.0

[a] Inoculated July 24.
[b] Average per cent infection all varieties upper surface inoculations 53.8, lower 92.3.

Inconspicuous lesions were noticed on some of the inoculated leaves after 8 and 9 days, respectively; therefore, under favorable weather conditions, such as light rainfall and moderate temperatures, which prevailed during these experiments, the period of incubation is perhaps 6 or 7 days.

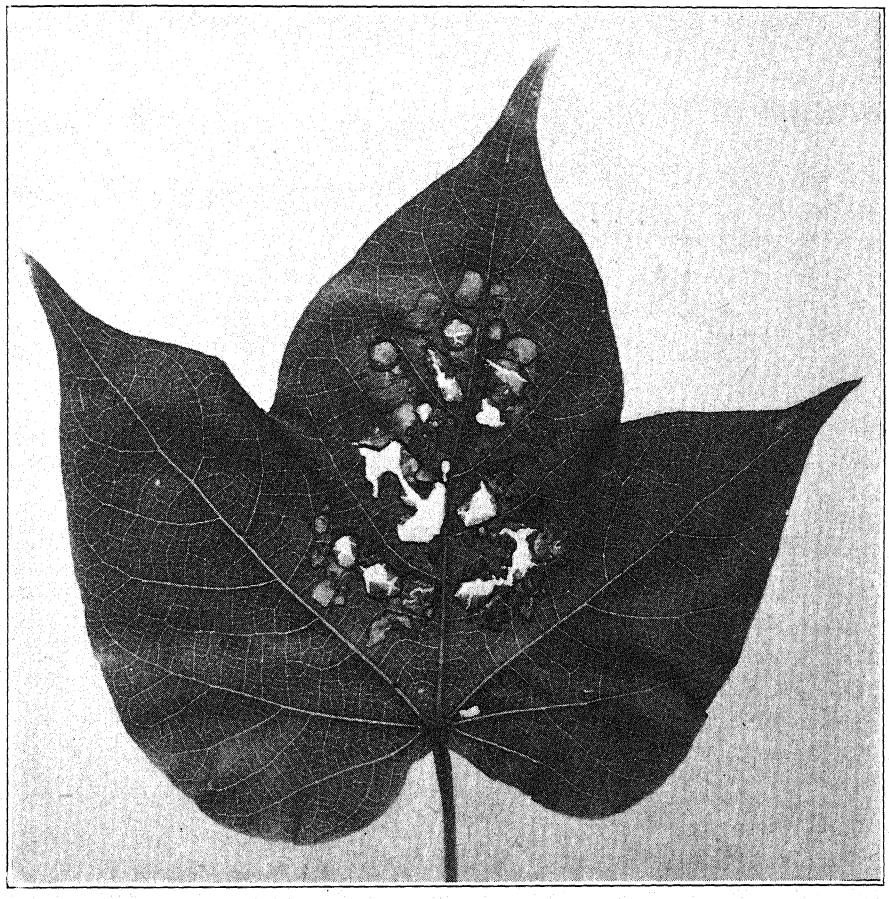


FIG. 2. Cotton leaves artificially infected with *Rhizoctonia* by placing sections of agar cultures of the fungus on the uninjured leaf surface. Inoculated July 26. Photographed August 3. Variety—Deltapine.

DISCUSSION

Although *Rhizoctonia solani* is known to cause "damping off" and "sore shin" of cotton seedlings (1) and sometimes persists when cold, wet weather continues through late spring, causing injury to root systems and stems of older plants (4), this is the first report, as far as the writer is aware, of leaves of mature cotton plants being attacked by a species of *Rhizoctonia*.

In studying possible sources of infection and means of dissemination of this fungus to the leaves of large cotton plants, a search was made of all other vegetation growing in close proximity, such as grasses and weeds

None of these hosts, however, gave evidence of being a carrier of the disease. Also, none of the cotton plants showed the Corticium stage of the fungus, thus eliminating probable spread by basidiospores. It would seem, therefore, that infection of the leaves may have occurred through dissemination of infested soil particles or debris, by wind, or other agencies. These points are still under investigation.

SUMMARY

A leaf spot of cotton, hitherto unreported and caused by the Rhizoctonia fungus, occurred at Baton Rouge, Louisiana, during the summer of 1943. It developed in mid-July on Deltapine plants, and later was found in other near-by fields on other varieties, including Coker and Delfos.

Leaf symptoms are distinctive and are described herein.

The fungus was readily isolated from infected leaves and its pathogenicity established through inoculation experiments. Under conditions favorable for infection, such as cloudy weather and moderate temperatures, the period of incubation was estimated to be approximately 6 or 7 days.

Cultural studies of this leaf-spot *Rhizoctonia* indicate that its morphology and growth characteristics are similar to and probably identical with the common soil fungus, *Rhizoctonia solani* (*Corticium solani*). Although certain possibilities present themselves, the exact manner by which it is disseminated to the leaves of mature cotton plants is unknown.

While the disease was frequently found attacking large leaf areas, sufficient to cause shedding, it has not been found yet to be of serious economic importance.

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SOME WAYS BY WHICH NUTRITION MAY AFFECT SEVERITY OF DISEASE IN PLANTS[1]

G. M. SHEAR AND S. A. WINGARD

(Accepted for publication January 27, 1944)

The purpose of this paper is to call attention to some experimental evidence obtained by other workers, and by the writers, that brings out possible relationships between certain unbalanced conditions in the mineral nutrition of a host plant and the severity of attack by certain vascular pathogens. That the nutrition of plants affects their susceptibility to certain diseases has been shown by numerous investigators and their findings have been reviewed by Wingard (8). In many instances the effect of nutrition seems to result from its effect on the general vigor of the plant. In other cases deficiencies of certain elements affect the susceptibility of a plant to a disease, while deficiencies of other essential nutrients have no apparent effect, thus showing that other factors are involved in such cases. These effects are not always easily explainable, although their pathological, as well as physiological, implications are of unquestioned importance.

The findings of Spencer and McNew (7) regarding the effects of nutrition on the susceptibility of sweet corn to *Phytomonas stewarti* show very clearly certain ways in which nutrition affects the development of a disease. They found that seedlings dwarfed by high concentrations of nitrogen, phosphorus, or potassium were more severely infected than those grown at concentrations more conducive to rapid growth, and that seedlings deficient in either nitrogen or phosphorus were only slightly infected whereas potassium-deficient seedlings were severely infected. Regarding the effect of potassium, they state that the disease was more severe in seedlings deficient in potassium than in those supplied with low concentrations of potassium and that this difference in severity may possibly be explained as follows: The pathogen may have a low potassium requirement and may cause considerable injury when inoculated into seedlings weakened by a deficiency of potassium. However, with the addition of small amounts of potassium, the seedlings start normal growth, and in so doing may become more resistant.

As a result of further study on the effect of nitrogen on the corn wilt organism, McNew and Spencer (4) showed that the amount of nitrogen in the tracheal sap of maize directly affects the growth of *Phytomonas stewarti*. This direct effect they state is probably attributable to the fact that the bacterium lives almost entirely in the tracheal tubes during the early stages of its invasion and, therefore, depends upon the materials in the transpiration stream for its sustenance. They present conclusive evidence, also, that the corn wilt bacterium depends on inorganic nitrogen in the tracheal sap for its parasitic existence.

[1] Published with the approval of the Director, Virginia Agricultural Experiment Station, as Scientific Paper No. 120.

The recent findings of Shear (6) regarding the effects of different amounts of fertilizer on the nutrient content of the conductive tissue of tobacco explain more fully the results obtained by Spencer and McNew and also throw some light on other ways by which nutrition may affect the susceptibility of a plant to disease. Increasing the amount of a nutrient in the fertilizer generally increased the amount of that nutrient in the conductive tissue. A deficiency of an element in the soil showed a corresponding decrease in the amount of that element in the conductive tissue of the plant.

However, the deficiency of a particular element sometimes resulted in a marked increase of the relative amounts of other elements in the conductive tissue, and this may be of importance in explaining the effect of a nutrient deficiency on the severity of infection by certain vascular parasites. When nitrogen, for example, was left out of the fertilizer, nitrate nitrogen in the conductive tissue was found to be very low, while phosphorus was found to be high and potassium not significantly affected. These findings agree with those of Carolus (1), who worked on certain truck crops and indicate that when nitrogen is deficient, protein formation is interfered with and the phosphorus that would otherwise be used in making protein compounds is accumulated in the conductive tissue. Apparently, however, the degree of nitrogen starvation was not great enough to interfere appreciably with carbohydrate metabolism in which the postassium was utilized.

In the tobacco experiments, when phosphorus was left out of the fertilizer, phosphorus in the conductive tissue was found to be very low, but the amount of nitrates and potassium was not affected thereby. These findings differ from those of Carolus (1), and Eckerson (2), working with tomatoes, who found that there was an accumulation of nitrate nitrogen in phosphorus-deficient plants. Eckerson explains that there is a decrease in the reductase activity in phosphorus-deficient tomato plants, which inhibits the utilization of nitrates. MacGillivray (3), on the other hand, found that there was an increase in the percentage of nitrogen in phosphorus-deficient tomato plants but that it was confined to water-soluble forms other than nitrates. These apparently conflicting results may be explainable on the basis of the degree of phosphorus deficiency obtained in the different studies. The phosphorus requirements of the various plants studied may have varied also.

When potassium was left out of the fertilizer mixture used in the tobacco experiments, potassium in the conductive tissue was found to be low, while phosphorus and nitrate nitrogen were high. Carolus (1) mentions the increase in nitrate nitrogen when potassium is deficient, but does not mention any effect that it has on the amount of phosphorus. Nightingale, Schermerhorn, and Robbins (5) found that a lack of potassium in tomato, beet, and lettuce plants retards the synthesis of organic nitrogen from nitrates. This might explain the high amounts of both nitrate and phosphorus found in the conductive tissue of tobacco. If the nitrates were not converted under

conditions of potassium deficiency, the utilization of phosphorus would be impeded the same as it is when nitrogen is deficient.

The preceding findings provide a basis for a logical explanation of the increased severity of wilt infection of corn seedlings deficient in potassium. It would appear, therefore, that the increased wilt severity is due to an increased rate of bacterial multiplication caused by the increase in nitrate nitrogen in the conductive tissue, resulting from the potassium deficiency. When potassium deficiency is less severe, more of the nitrate in the corn plant would be utilized by the plant itself, thus reducing the amount in the conductive tissue available for the growth of the wilt organism. This in turn would lessen the severity of the effects caused by the parasite.

Such a direct relationship between the nutrition of the host and the parasite as that suggested above would not occur in many instances, as most parasitic organisms require more complex nitrogen compounds for their nutrition. Assuming our interpretation to be correct, if the severity of infection caused by a disease-producing organism were found increased by addition of an excess of nitrogenous fertilizer, but not increased by a deficiency of potassium, the inference would be that the pathogen probably could not directly utilize nitrate-nitrogen within the host. It might perhaps indicate that the severity of infection was increased either by the succulence of the host tissue or more probably by the presence of an abundance of some more complex nitrogen compound, essential to the growth of the parasite, which was being produced in excess of the requirements of the host.

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COMPARATIVE STUDIES OF TWO CARROT LEAF DISEASES[1]

W. J. HOOKER[2]

(Accepted for publication January 29, 1944)

INTRODUCTION

In Wisconsin, as well as in many other areas, carrots (*Daucus carotae* L.) are attacked by 2 leaf-spotting fungi, *Cercospora carotae* (Pass.) Solheim and *Macrosporium carotae* Ellis and Langlois. It has been noticed in Wisconsin and has been reported in Massachusetts (1) that the seasonal cycles of these diseases differ from each other. *C. carotae* occurs most commonly during July and early August in Wisconsin, whereas *M. carotae* develops in severity during August and throughout September. This study was initiated to determine whether the difference in seasonal cycle could be explained on the basis of the response of the 2 fungi to temperature or to other factors.

METHODS

Single-spore cultures of *Macrosporium carotae* did not sporulate on potato-dextrose agar, and as a result a medium containing 6 per cent fresh carrot leaves and 2 per cent agar was used for spore production. It was later shown that 2 per cent water agar induced sporulation equally well. Both media supported only meager vegetative growth. *Cercospora carotae* was grown on a medium prepared with carrots, cucumber, and agar described by Mrak, Phaff, and Douglas (2).

Seedling carrots of the Danvers Half Long Variety in the 4- to 6-leaf stage were used in greenhouse studies with the exception of 1 experiment in which leaves developed from storage roots of the Chantenay variety were used. Leaves were inoculated by spraying with a spore suspension. The plants were held in moist chambers for 48 hours, and then they were returned to the greenhouse bench. The houses in which inoculated plants were incubated were held at constant temperatures of 16, 20, 24 and 28° C., respectively. Each inoculated leaf was placed in 1 of 4 classes designated as healthy and slightly, moderately, or severely diseased, respectively. From such data a disease index was calculated in which the 4 classes were given equal weight. Thus the index for any given set of plants might vary from 0, indicating all were healthy, to 100, in which all were severely diseased. In certain cases the data were treated by the analysis of variance method. The writer is indebted to Dr. J. H. Torrie for advice in connection with this phase of the study.

SYMPTOMS OF THE TWO DISEASES

Differences in symptoms of the 2 diseases have been described by Doran

[1] The writer is indebted to Dr. J. C. Walker for advice during the course of this investigation and in the preparation of the manuscript.

[2] Formerly Instructor in Plant Pathology, University of Wisconsin; at present Research Associate in Botany, Iowa State College, Ames, Iowa.

and Guba (1) and those of *Cercospora carotae,* described and illustrated by Thomas (3). The distinction between the diseases is best shown in figure 1.



FIG. 1. A, *Macrosporium carotae;* and B, *Cercospora carotae* on leaves of carrot. *M. carotae* generally produces dark, irregularly shaped lesions whereas those produced by *C. carotae* have a light-tan-colored center and tend to be more nearly circular in shape.

The spots caused by *C. carotae* are, in general, more nearly circular than those caused by *Macrosporium carotae.* Leaf tissue is invaded more rapidly

by the latter fungus resulting in more irregularly shaped lesions which may quickly involve the leaflet tip or the entire leaflet. The leaf spot caused by *C. carotae* is generally a light tan color, especially if the disease has developed during periods of relatively low humidity, as was the case with the leaf shown in figure 1; whereas, if development has occurred in a moist atmosphere, the entire lesion may be dark colored and not so nearly circular, as illustrated by Thomas (3). Lesions produced by *M. carotae* are generally dark-brown to black, and chlorosis of surrounding tissue is considerably more prominent than in the case of spots caused by *C. carotae.* Both fungi attack the petioles, producing elongate lesions that often result in death of the entire leaf. Petiole lesions in the case of *C. carotae* often have a more or less light-colored center, whereas those caused by *M. carotae* are dark in color.

TEMPERATURE RELATIONS IN PURE CULTURE

Throughout the investigation spore germination and growth responses in Petri-dish cultures were determined on potato-dextrose agar. Spore suspensions were placed in Petri dishes at room temperature in experiments 1 and 2, whereas in experiment 3 the spore suspension was added to plates previously brought to incubator temperature. Observations were made at the end of about 3 hours with *Macrosporium carotae* and at the end of 24 hours with *Cercospora carotae.* The percentage of germination at different temperatures is shown in table 1. In the case of *M. carotae* germination was practically complete over a range of 20 to 37° C. during the 3-hour period of incubation. Slight reduction was noted at 16°, marked reduction at 12°, and no germination at 8 and 4°. Observations after 1- and 2-day incubation showed good germination at both 4 and 8°, while at 37° good germination occurred with no further mycelial development. Practically complete germination of *C. carotae* occurred at 20 to 32°; it was slightly reduced at 16°, and markedly so at 8°. No germination was observed at 4°, but, after a week, short germ tubes had developed, while during this interval none had

TABLE 1.—*Effect of temperature on germination of spores of Macrosporium carotae and Cercospora carotae*

Temp.	*Macrosporium carotae*				*Cercospora carotae*			
	Experiment number			Average	Experiment number			Average
	1	2	3		1	2	3	
°C.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
4	0	0	0	0	0	0	0	0
8	0	0	0	0	8	8	12	9
12	36	12	72	40	50	91	26	56
16	91	10	88	63	92	91	84	89
20	92	85	100	92	100	96	98	98
24	100	79	95	91	100	97	100	99
28	100	93	100	98	98	99	100	99
32	100	100	100	100	98	93	100	97
37	.....	96	86	91	0	0	0	0

developed at 37°. These results are in general accord with those of Doran and Guba (1) and Thomas (3).

For studies of growth, cultures in dishes were inoculated in the center with a heavy suspension of spores in the case of *Cercospora carotae* and with a 5-mm. disk of mycelium and agar in the case of *Macrosporium carotae.* Measurements of colony diameter were made at the end of 1 week. Relative mycelial growth of both fungi in culture is shown in figure 2. Both fungi showed a distinct growth optimum at 28° C.

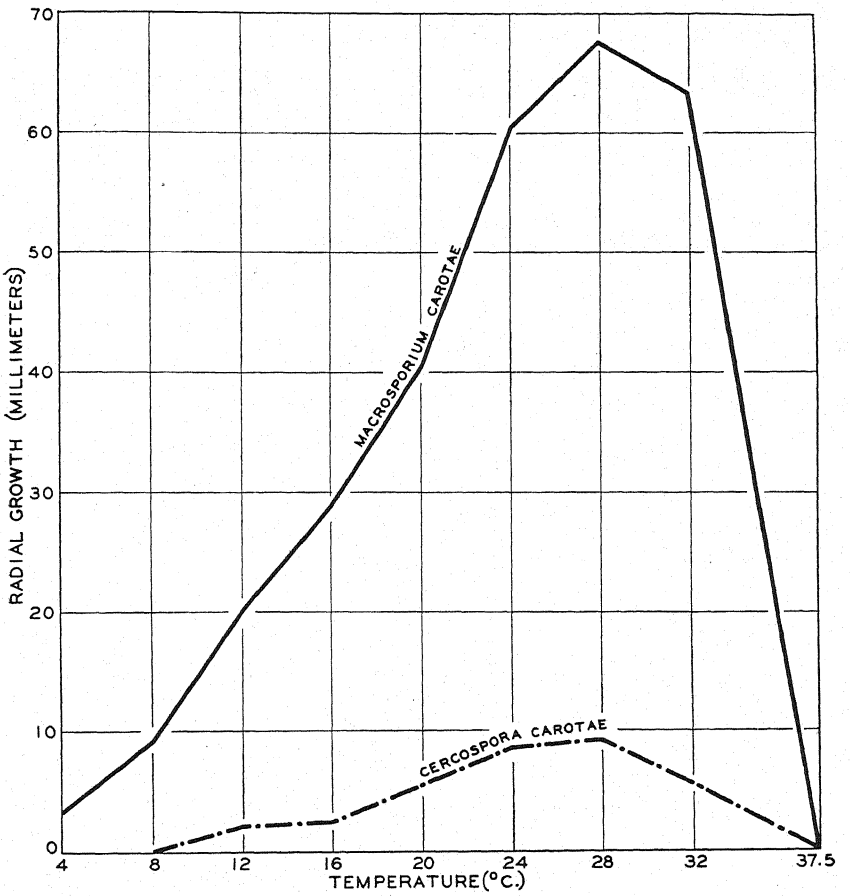


FIG. 2. Temperature relations of *Macrosporium carotae* and *Cercospora carotae* as indicated by colony diameter after incubation for 1 week on potato-dextrose agar at temperatures indicated. Graph drawn by Eugene Herrling.

INFLUENCE OF TEMPERATURE ON DISEASE DEVELOPMENT

In order to distinguish between the effect of temperature on infection and on subsequent disease development, 2 distinct series of inoculations were carried out. In the first case, the temperature of inoculation was maintained the same as that of the house in which disease development was to take place by putting individual moist chambers over the plants in the respective tem-

perature houses. In the second case, plants were inoculated in a common moist chamber and then removed to their respective temperature houses. The optimum temperature for seedling development of Danver's Half Long carrot was about 24° C.

The results of inoculation experiments in which inoculation and subsequent disease development occurred at the same temperature are shown in table 2. Markedly slower disease development by *Macrosporium carotae* occurred at the low temperatures. In all trials the disease developed more slowly at 16° C. than at 20°. Although this difference was not significant at the 5 per cent level, it was marked. Significant differences were obtained when disease development at 16 or at 20° was compared with that at 24 or

TABLE 2.—*Disease development by Macrosporium carotae and Cercospora carotae on leaves of carrot at temperatures indicated*

Organism	Temp., °C.	Disease index				
		Replication number				Average
		1	2	3	4	
Macrosporium carotae	16	28	11	6	10	13.75
	20	53	25	13	15	26.50
	24	51	44	49	59	50.75
	28	55	25	47	52	44.75
Cercospora carotae	16	15	12	12	2	10.25
	20	24	32	37	12	26.25
	24	41	43	52	53	47.25
	28	42	53	67	53	53.75
Difference required for significance (19:1)						15.6

at 28°. A slight but not significant reduction in disease occurred in 3 of the 4 trials between 24 and 28°. Although chlorosis did not develop consistently, both diseases showed greater chlorosis at 16 and 20° than at 24 and 28°. In general, infection by *M. carotae* resulted in more chlorosis than was the case with *Cercospora carotae*.

The trends with *Cercospora carotae* were, in general, more clearly defined than are those shown by *Macrosporium carotae*. Disease development increased progressively with temperature. Significant differences at the 5 per cent level were obtained between 16 and 20° C. and between 20 and 24°. The difference between 24 and 28° was not significant.

When another series was run in which all the plants were inoculated at 20° C., and the plants then incubated at 16, 20, 24, and 28°, the same relation of temperature to disease development maintained. It may be concluded, therefore, that there is little or no difference to be found between the 2 fungi in their response to temperature as measured by spore germination, growth in pure culture, or in the progress of pathogenesis at constant temperature levels. The difference in seasonal cycles of the 2 diseases must, therefore, be based on other factors.

RELATION OF AGE OF LEAF TO DISEASE DEVELOPMENT

It was observed during the temperature studies that *Cercospora carotae* developed most severely on the younger leaves, whereas *Macrosporium carotae* showed a preference for the older leaves. Doran and Guba (1) inoculated excised carrot leaves with *M. carotae.* After 3 weeks they observed an average of 24 infection points on old leaves for each point of infection on young leaves. They reported that young leaves were susceptible to *C. carotae,* but apparently they did not observe that young leaves were more susceptible than old leaves. In this investigation, plants in the 4-leaf stage were used. Leaves were numbered from 1 for the youngest to 4 for the oldest. The results given in table 3 show the striking difference between

TABLE 3.—*Influence of leaf age upon development of carrot leaf blight and leaf spot*

Organism	Leaf No.[a]	Disease index						
		Replication number						Average
		1	2	3	4	5	6	
Macrosporium carotae	1	6	0	0	12	19	25	10.3
	2	37	12	31	12	50	31	28.8
	3	56	37	37	50	37	75	48.7
	4	69	50	69	62	87	94	71.8
	Difference required for significance (19: 1)[b]							28.0
Cercospora carotae	1	75	62	75	37	58	75	63.7
	2	75	19	37	31	50	58	45.0
	3	37	19	31	25	17	42	28.5
	4	19	19	37	25	8	37	24.3
	Difference required for significance (19: 1)							26.7

[a] Leaves numbered from the youngest to oldest on seedling carrots.
[b] The variances within treatments were homogeneous by Bartlett's test for homogeneity.

the 2 fungi. *M. carotae* increased very decidedly in severity from the youngest to the oldest leaf while with *C. carotae* the reverse was true.

SUMMARY AND CONCLUSIONS

The leaf blights of carrot have been studied under controlled conditions to determine what might be the underlying causes of the striking contrast in season cycles of the 2 diseases. Spores of both fungi germinated over a wide range of temperature with optimum for both at 28° C. Low temperature (16° C.) retarded disease development with both, while there was an increase in disease index with increase in temperature with both up to 24° C. It is thus apparent that temperature has little to do with determining the early-seasonal severity of *Cercospora carotae* and the late seasonal development of *Macrosporium carotae.*

When age of leaf was studied in relation to disease index, a wide contrast was to be found. *C. carotae* was most severe on young leaves, and patho-

genicity decreased markedly on older leaves at time of inoculation. *M. carotae*, on the contrary, was least pathogenic on young leaves and most severe on old leaves. This difference in the relative resistance of the host leaves to the 2 fungi may account in large measure for the fact that one disease is at its peak in mid-season when high temperature and young foliage favor it, while the second finds a more congenial substrate later in the season as more leaves approach maturity even though temperatures at that time are slightly less favorable.

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PHYTOPATHOLOGICAL NOTE

The Nomenclature of the Broomcorn Millet Smut Fungus.—In the interests of stability of nomenclature the Federal Department of Agriculture follows the International Rules of Botanical nomenclature in its publications. This will bring about changes in a number of fungus names heretofore in common use and this is particularly true in the case of names for the cereal smut fungi. However, only in one instance has it been found necessary to set up a new binary name and that involves the broomcorn millet smut fungus, now known in American practice as *Sphacelotheca panici-miliacei* (Pers.) Bub.

Persoon in his *Synopsis Fungorum* (1801), which is the starting point for the nomenclature of the smut fungi, lists the millet smut fungus as a subspecies, *panici-miliacei,* of his *Uredo segetum,* and this is the name which has been more commonly used as a specific epithet. However, Schlechtendahl in 1824 validly published *Caeoma destruens* as a name for this species, citing Persoon's name, and thus *destruens* becomes the first legitimate specific epithet available under the provisions of section 11, article 58, of the rules. It does not appear that transfer to the genus *Sphacelotheca* has been made previously and in order that the name may be available, the necessary new combination is here proposed, with pertinent synonymy appended.

Sphacelotheca destruens (Schlecht.) comb. nov.

Uredo segetum subsp. *panici-miliacei* Pers. Syn. Fung., p. 224. 1801.

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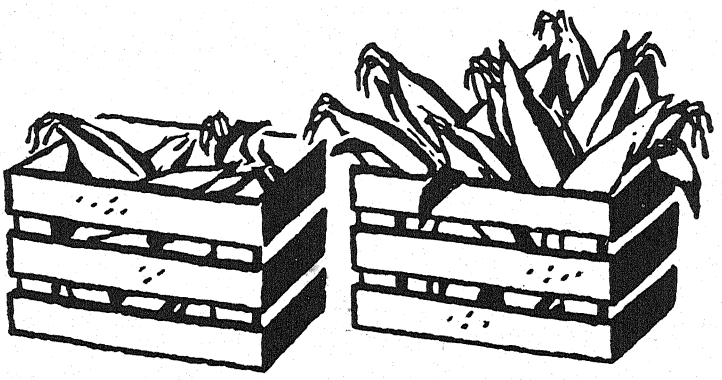
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—JOHN A. STEVENSON and A. G. JOHNSON, Bureau of Plant Industry Station, Beltsville, Maryland.



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BASAL ROT OF TOMATO[1]

J. K. RICHARDSON[2] AND G. H. BERKELEY[3]

(Accepted for publication October 25, 1943)

INTRODUCTION

A hitherto unreported fungous basal rot on tomatoes appeared in a greenhouse in the London, Ontario, district in 1936 and 1937, and in another in 1942. Though the disease is apparently uncommon in Ontario, the fact that the crop may be reduced as much as 50 per cent warrants the publication of a report at this time, even though the identity of the causal fungus has not yet been established.

SYMPTOMS OF THE DISEASE

The first apparent symptom is a marginal chlorosis of the leaves, most severe at the base of the plant, and progressing upwards. Affected leaves become necrotic, wither and die, resulting in a premature defoliation, and dwarfing of affected plants (Fig. 1, B), with a distinct tendency towards wilting during periods of excessive transpiration.

The foliar symptoms result from an infected stalk and root system, in which the cortical tissue of the basal section of the stalk and roots, especially the laterals, may be entirely disintegrated. On the larger roots, especially of older plants, are found brown necrotic lesions, and characteristic "cankers" in which the cortical tissue is swollen and cracked (Fig. 1, A). In addition to the definite brown necrotic lesions on the roots, many infections appear as a brown flecking, which produces a russetting of the epidermal tissues.

ISOLATIONS

Isolations were made from lesions of all types and from roots of all sizes. This material was thoroughly brushed in water to remove all adhering soil particles, cut into pieces $\frac{1}{8}$ to $\frac{3}{8}$ inches long, washed in running water for at least 3 hours, then pressed into solidified potato-dextrose agar in culture dishes, and allowed to incubate at room temperature. Hyphal-tip transfers were made from the mycelial growth during a subsequent 2-week period in order to isolate as many fungi as possible.

Over 1700 isolations were made from upwards of 700 plantings of all types of root lesions on plants of different ages growing in the greenhouse at different seasons of the year. Species of 10 identified and as many unidentified genera of fungi were obtained from these isolations, but those appearing in the largest numbers were *Trichoderma* spp., *Fusarium* spp., *Cylindrocarpon* spp., *Pythium* spp. and an unidentified fungus, hereafter designated as *TR*. Although the organism *TR* did not appear more fre-

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[3] Pathologist-in-charge.

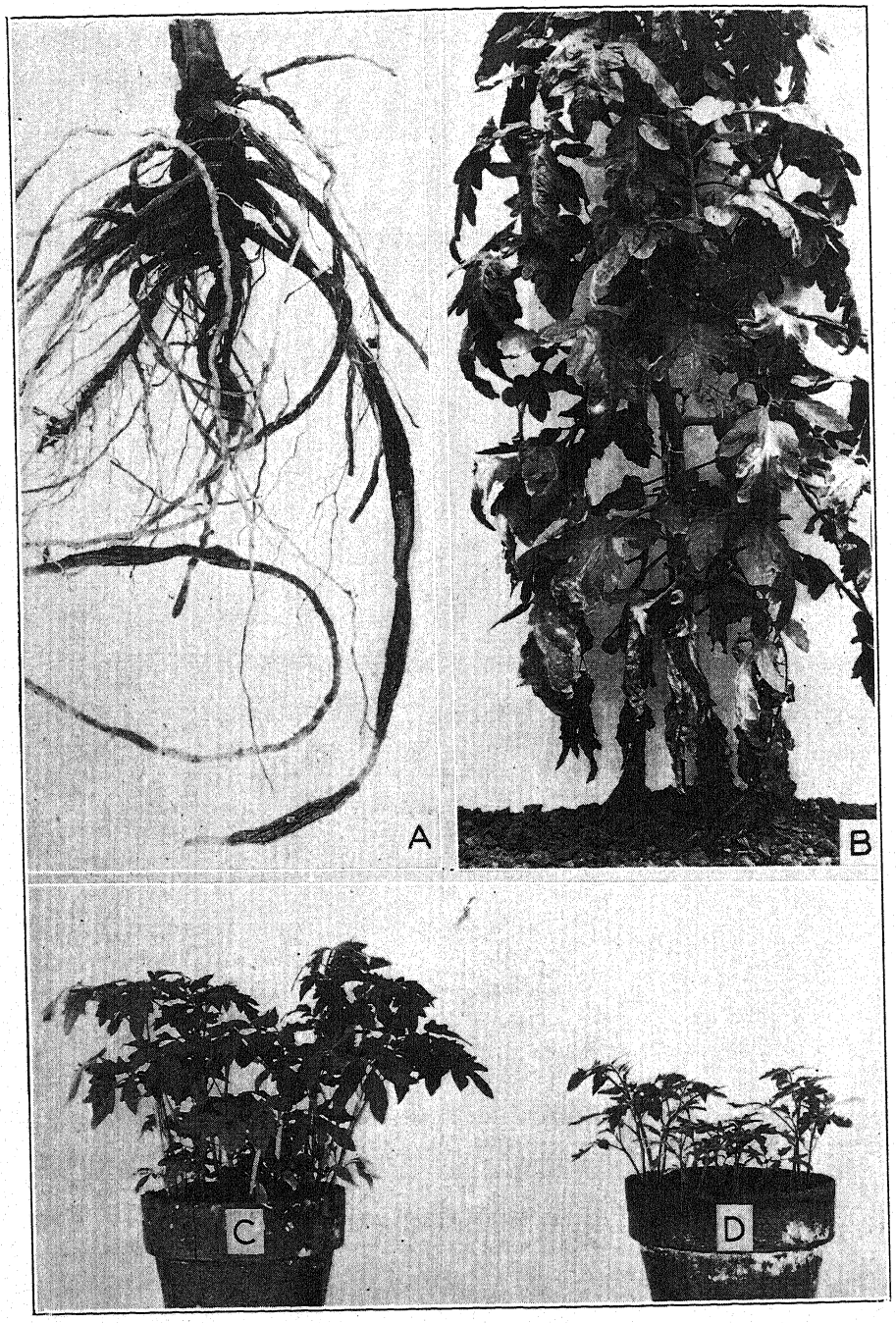


FIG. 1. A. Roots of an infected tomato plant showing brown cankerous swellings. B. Typical yellowing, wilting and dying of the lower leaves of an infected plant. C and D. Seedlings growing in naturally infected soil (C) after and (D) before sterilization with steam.

quently than the other species isolated, it did appear more consistently from the various types of plated material, thus suggesting a direct relationship with the disease.

INOCULATIONS

A series of inoculations using naturally contaminated soil, (1) in its original condition, (2) steamed, and (3) steamed, then inoculated with oat cultures of *TR*, were carried out at 5 constant-temperature ranges as follows, 12–14° C., 15–17° C., 18–20° C., 22–24° C., and 26–28° C. The artificial growing conditions made the foliage symptoms somewhat inconsistent, but an examination of the roots revealed that the disease was more severe in the lower temperature ranges and somewhat less pronounced in the artificially inoculated than in the naturally infected soil. All control plants growing in the steamed soil had clean, white, healthy roots. Although typical root rot developed, there was an absence of the large, cracked swollen lesions associated with the disease under normal greenhouse conditions, suggesting that other organisms might be involved or that there might be a relationship between size of root and size of lesion.

Accordingly, a second series of inoculations was made to test the pathogenicity of the 5 most frequently isolated fungi. Five isolates of each of these fungi were grown on sterilized compost moistened with a 2 per cent solution of dextrose in water. After incubation at room temperature for 4 weeks the 5 isolates of each fungus were mixed together. In turn these composite cultures were mixed in all possible combinations. Small quantities of this mixed inoculum were then added to pots of contaminated soil that had previously been thoroughly steamed. This procedure made a series of 31 differently inoculated soils in addition to uninoculated steamed and non-steamed contaminated soil. Each lot was divided into 4 pots, which were planted with tomato seedlings and kept in the greenhouse.

Throughout the duration of the experiment no records were taken on the growth of the plants because at no time was the variation between the plants in the differently inoculated soils greater than that between replicates of a similarly treated group.

Upon examination of the roots of one series of plants on June 16, 27 days after inoculation, it was noted that, though there was some variation in bulk and extent of discoloration, there was no consistent correlation between their condition and the inoculum the soil had received; even control plants growing in naturally contaminated soil had comparatively clean and healthy-appearing roots. The roots of a second series of plants were examined on July 15, 56 days after being inoculated, but again no significant variations could be observed.

Final observations were taken on the remaining two series of plants on October 6, 109 days after being inoculated; at this time the results were significant. Although there were few roots that showed the conspicuous swollen, cracked lesions, due possibly to their small size and confinement in the pots, there was a great variation in the number that showed browning

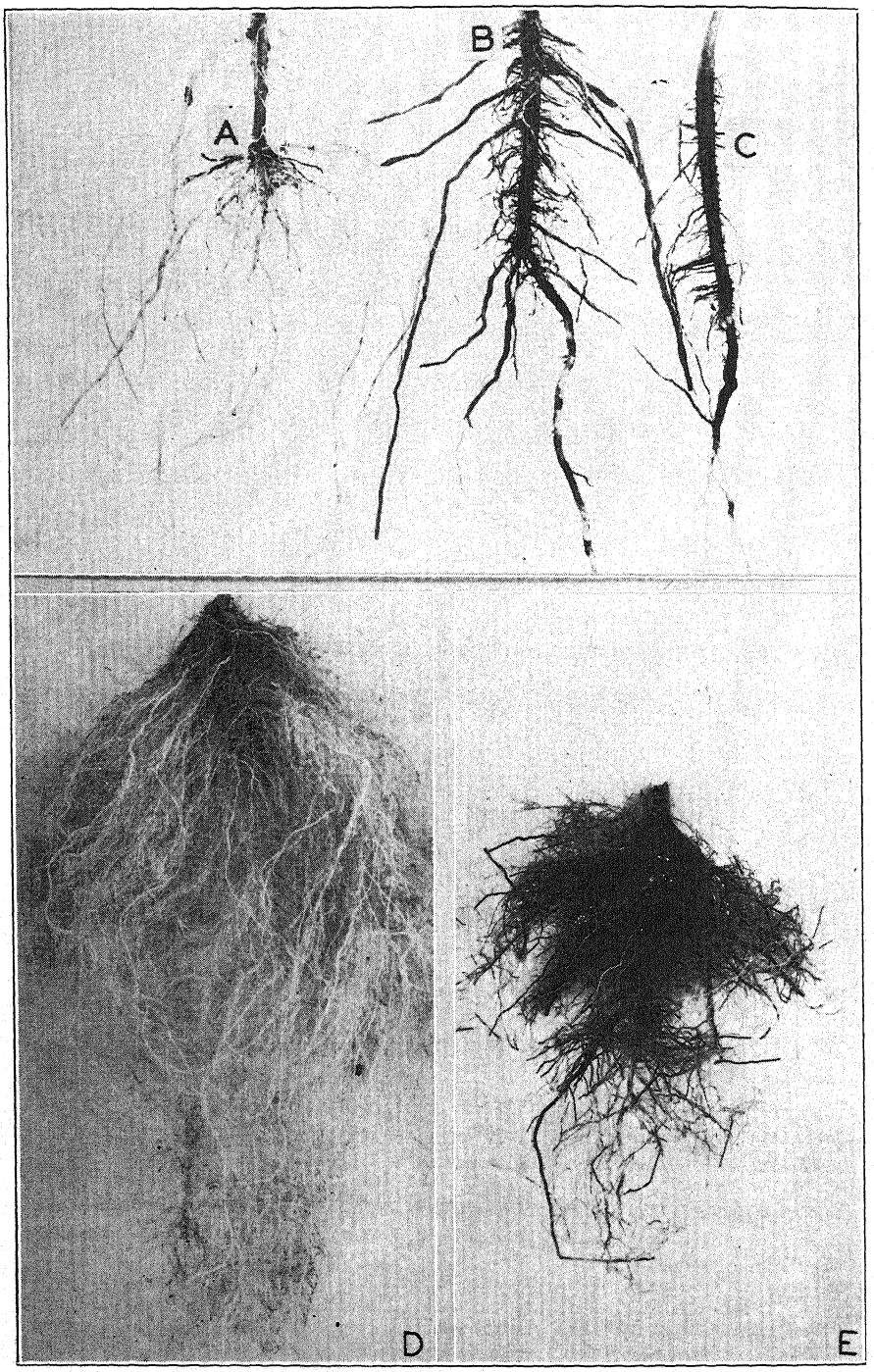


FIG. 2. A to C. Roots of plants growing in the greenhouse, A in sterilized soil and B and C in nonsterilized soil. D. Roots of tomato plant in pot of steamed compost. E. Roots of plant grown in pot of compost steamed then inoculated with soil culture of *TR*.

and necrosis. Roots growing in contaminated steamed soil were white and fibrous with only slight discoloration (Fig. 2, D). The roots on controls growing in similar nonsteamed soil, however, were greatly reduced in bulk, the larger ones severely browned and necrotic, with most of the smaller ones disintegrated.

All root systems of plants in soil inoculated with isolates of a single fungus other than *TR*, or in combinations where *TR* was not present, were, for the most part, comparable in bulk and appearance to the controls in steamed soil (Fig. 2, D). However, where *TR* had been used in the inoculum, either alone or in combination with other species, the roots were severely diseased (Fig. 2, E), and, in some cases, in even worse condition than those growing in nonsteamed contaminated soil. Reisolations of *TR* were obtained readily from these roots.

These results prove not only that *TR* is capable of causing the disease, but also that it was the only species of the 5 tested that had more than a minor detrimental effect on tomato roots. All others were probably secondary invaders and bear no significant connection with the disease.

DESCRIPTION OF THE PATHOGEN

Though the causal fungus grows slowly, it can be readily isolated and cultured on various types of media. However, since no fruiting bodies have been found, identification has not yet been possible.

In an attempt to induce sporulation, cultures have been grown on both solid and liquid potato, prune, Lima-bean, and corn-meal media alone and with varying quantities of dextrose; malt and beef extract with and without peptone and potato-dextrose, and corn-meal agars with various pH values; on potato plugs and stems, tomato stem and root material, in various types of soils and soil solutions, and on several synthetic media. Cultures were held at various constant temperatures at variable room and basement temperatures in light and in darkness, removed from cold to warm locations and *vice versa,* and subjected to ultraviolet light for varying lengths of time. Both cultures and diseased roots have been exposed to drought, excessive moisture, freezing, alternate freezing and thawing, drying and wetting, and refrigeration at various stages of growth, but all to no avail.

Failing in our attempts to identify the organism, cultures were sent to mycologists at Ottawa, Cornell University, and the Imperial Mycological Institute, England, but all reported they were unable to identify it.

The causal fungus is a nonsporulating, dark-colored, slow-growing organism, spreading from 3 to 5 cm. on potato-dextrose agar in 10 days at 23–24° C., and covering the surface of a 10-cm. Petri dish in from 3 to 4 weeks. Aerial mycelial growth is tight-cottony, smooth, or slightly tufted, raised to 0.3 to 0.5 cm. above the surface of the medium, and light-gray, at times tinted with subdued pink. With age, the mycelium collapses to the surface of the culture, becoming moist and shiny. The reverse of the colony is zoned from a central point, and varies in color from grayish-brown to greenish-black, depending on the intensity of pigmentation of the medium, and is bordered by a narrow margin of light growth. Young mycelium is 2–4 μ thick, sparsely branching with few septa, and has a tendency to form narrow strands of closely packed paralleled hyphae running through the thallus. With age, it becomes darker colored, coarser, from 4 to 8 μ thick, becoming very closely septated with constrictions mostly at, but occasionally between, the septa, giving the appearance of chains of irregularly shaped, thin-walled chlamydospores.

CONTROL

Though it was known that sterilization of the soil by steam was effective in controlling basal rot (Fig. 1, C), it was desirable to test the effect of disinfection by chemicals, since steam is not available for sterilization purposes in all greenhouses.

Accordingly, the soil in a section of the laboratory greenhouse was inoculated by mixing with it a quantity of the contaminated soil from a greenhouse in London, Ontario. A test tomato crop grown in this mixed soil became severely affected with basal rot, indicating successful inoculation of the soil.

Accordingly, areas 8 × 9 feet were treated with steam, chloropicrin, and Formalin, respectively, while a section twice as large was left untreated as a control. The chloropicrin was applied with a Larvajector in dosages of 2½ cc., 6 to 8 inches deep and 10 inches apart, and Formalin was used 1 part in 50, at the rate of ¾ of a gallon to each square foot of soil. After treatment with Formalin, the soil was covered for 3 days with moist paper, while a gas-proof paper was placed over the chloropicrin-treated soil for a similar period. At the time of treatment the temperature of the soil at a depth of 4 inches was 76° F. After the prescribed elapsed time, tomato plants of the Grand Rapids variety were planted in all plots, and records were taken as to height and crop. In all, 22 pickings were made between June 23 and August 12, 1941, as recorded in the following table.

TABLE 1.—*Yield of tomatoes June to August, 1941*

Treatment	No. of plants	Weight of fruit in pounds	Weight per plant
Steam	14	149	10.6
Chloropicrin	14	139	9.9
Formalin	14	143	10.2
Untreated	28	154	5.5

When harvesting commenced, the plants in the untreated section were much smaller, lighter in color, and only half as tall; and those in the Formalin section were 6 to 8 inches shorter than the plants in the steam and chloropicrin-treated areas. An examination of the roots showed no visible signs of the disease in the steam- or chloropicrin-treated soils (Fig. 2, A), whereas a trace was present in the Formalin-treated soil, and severe infection in the untreated control (Fig. 2, B and C).

After this crop had been harvested, the plants were removed, and the soil from all plots was thoroughly intermixed. In the fall of 1941 the experiment was repeated, using similar steam and Formalin treatments; but the chloropicrin injections were reduced from 2½ to 2 cc., and the gas was retained in the soil by keeping the surface of the soil wet for 3 days, instead of using the special gas-proof paper covering.

Though the plants were smaller in this experiment, the disease on the various plots was similar to that found in the first test. The average yield

TABLE 2.—*Yield of tomatoes from 18 pickings extending from February 12 to May 1, 1942*

Treatment	No. of plants	Weight in pounds	Weight per plant
Steam	15	40.5	2.7
Chloropicrin	15	48.0	3.2
Formalin	15	39.0	2.6
Untreated	20	24.0	1.2

from the two experiments covering a fall and a spring crop was 6.6 lb. per plant for steam sterilization, 6.5 for chloropicrin, 6.4 for Formalin, and 3.3 for the untreated control.

These results clearly indicated not only that basal rot reduces the yield, but that it can be satisfactorily controlled by soil sterilization with steam, chloropicrin, or Formalin, though Formalin is apparently the least effective of the three.

SUMMARY

A hitherto unreported basal rot of greenhouse tomato, caused by an unidentified fungus has been found in the vicinity of London, Ontario.

Isolations and inoculations have proved the pathogenicity of the causal organism.

The symptoms of the disease, consisting of defoliation of the lower leaves and a cortical rot of stalk and roots, and a description of the pathogen are given in detail.

The disease, which may reduce yield as much as 50 per cent, can be controlled by soil sterilization with steam, chloropicrin, or Formalin.

DEPARTMENT OF AGRICULTURE,
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THE UNIMPORTANCE OF TOMATO SEED IN THE DISSEMINATION OF VERTICILLIUM WILT IN CALIFORNIA[1]

B. A. RUDOLPH[2]

(Accepted for publication January 14, 1944)

Verticillium wilt of tomatoes was first reported in California by Rudolph (6) in 1926. He also advanced proof (6, 7) that certain other diseases so troublesome in the State, namely, "blue stem" of raspberries and "black heart" of stone fruits, are of identical origin and readily intercommunicable. That the disease, caused by the soil-borne fungus *Verticillium albo-atrum* R. and B., was not recognized in tomatoes in California at an earlier date by other workers may be attributed, in all probability, to the fact that its symptoms are so similar to those of Fusarium wilt, already established and well known in the State, that it was simply mistaken for it. Subsequent studies showed that most of the so-called Fusarium wilt in the central coastal counties of California, particularly in the San Francisco Bay Region, is actually Verticillium wilt, also that true Fusarium wilt ordinarily is of little economic importance there. The latter has been shown by Shapovalov and Lesley (8) to constitute a serious problem in other sections of California, however, including several coastal counties where both fungi may prove equally destructive in the same field and even in the same plants (10).

In 1939, Shapovalov and Rudolph (9) briefly described the Essar,[3] a Verticillium-wilt-resistant canning tomato produced at the University of California Deciduous Fruit Field Station at San Jose, with the financial assistance of and at the request of the Canners League of California, which organization had come to recognize the seriousness of the disease in the San Francisco Bay Region and attributed to it much of the decline in tomato production there, both from the standpoint of quantity and quality.

Not only canners but growers and seedsmen alike became interested in this seemingly new disease, especially the latter, since seed production is an important industry in California. Because of the suddenness with which all classes of tomato growers became aware of the prevalence of Verticillium wilt virtually everywhere in the State, it was only natural that the question should arise as to what role, if any, the seed plays in its transmission.

In 1932, experiments designed to throw light on the problem were started at the Deciduous Fruit Field Station at San Jose and concluded 10 years later. In 1934, Kadow (2) published the results of similar experi-

[1] Received for publication January 4, 1944. Much of the work reported in this paper was done under Work Projects Administration, Projects 50–1859—50–11992—50–12456–D.

[2] Associate Plant Pathologist in the California Agricultural Experiment Station.

[3] A technical description of the Essar tomato, together with data covering canning tests made annually for five years by the Canners League of California as well as physical and chemical tests made by the National Canners Association has been written but never published. These data show that in addition to its greater resistance to Verticillium wilt, the Essar very definitely excels the Early Santa Clara Canner, the variety most commonly used in California for canning, in the production of "solid pack" and other better grades of canned tomatoes.

ments from which he concluded (2, 3) the disease to be seed-borne. Because his results were at such great variance with those already obtained here, and because no other reports of similar work have appeared, it was considered inadvisable to discontinue the experiments until there could no longer be any reasonable doubt, under California conditions at least, as to whether the seed actually can constitute a real factor in the dispersal of the parasite.

THE PLAN OF THE EXPERIMENTS

Except for certain changes and simplification of technique described farther on, expedient to the production of a greater number of cultures, the general plan of the experiments was as follows:

In the fall, a representative sample, or even the entire crop, of sound, ripe tomatoes produced on one or more plants in the most advanced stage of Verticillium wilt[4] were clipped from the vines. The plants themselves were also dug up and cultures made from the main stems to prove the presence of the parasite. The routine procedure consisted of scrubbing a section of the main stem of each plant with a brush, disinfecting the same by immersing it in a mercuric bichloride solution 1/1000 for 5 minutes, after which it was washed with sterile distilled water, the usual precautions to insure asepsis being taken at all times. Plantings taken from the discolored vascular system were made in Petri plates of Czapeck's agar slightly acidified with lactic acid to suppress bacterial growth. At the same time, cultures were also made from the receptacles of each fruit, great care being taken in working them free by hand not to tear the flesh at the point of attachment to the tomato. Each receptacle was then clipped from the peduncle at the node, scraped with a scalpel to remove dead cuticle and dirt adhering to it, scrubbed with a brush and disinfected in the manner described. Then the receptacle and short length of peduncle attached to it were cut into 3 or 4 sections with flamed instruments and planted in a Petri plate of slightly acidified Czapeck's agar.

Each fruit was lightly sponged with a very weak solution of iodine in 95 per cent ethyl alcohol after which a conical plug, which included the point of stem attachment and most of the core, was cut out with a flamed scalpel. Three or 4 transverse sections of this plug were sliced off and placed in a Petri plate of slightly acidified Czapeck's agar. This phase of the experiment was designed to show to what extent the fungus, having reached the receptacle, is capable of invading the vascular system of the fruit, which is highly developed in the core. The tomatoes from which the

[4] In experiments conducted at the Deciduous Fruit Field Station to determine the relative resistance of many well-known varieties of canning and market tomatoes as well as hybrids of the same to Verticillium wilt, large numbers of plants are artificially infected as follows:

At the time the young plants are set in the field, a maximal slant culture of *Verticillium albo-atrum* R. and B. grown on Czapeck's nutrient agar in standard culture tubes, ¾ inch outside diameter and 6 inches in length, is scraped into the hole in direct contact with the roots of each. A 2% agar is used to make the medium tough and to facilitate its easy removal from the tubes. The fungus grows well upon Czapeck's agar and produces its micro-sclerotia over the entire surface in a continuous or unbroken crust. The test is severe, and few plants inoculated in this way escape infection.

cores had been removed, properly identified, were kept in sterilized jars in an electric refrigerator pending the outcome of the cultures.

Finally, cultures were made from the seed of each tomato, the core of which had yielded *Verticillium* in culture. Each fruit was squeezed gently, and as the seeds oozed out of the cavity made by the removal of the core, they were picked up with sterile forceps and planted in slightly acidified plates of Czapeck's agar, usually 8 seeds to a plate.

A minimum of 3 weeks was allowed to elapse from the time any culture was made until it was discarded if *Verticillium* had not developed earlier.

Results Obtained in 1932. In Table 1, the results obtained with the technique described above are given. A record was made of the discoloration observed, if any, in the vascular system of the receptacle and the core. All plants were severely diseased, and the fungus had been isolated from the main stems.

In this preliminary experiment no count of the seed cultured was made, but it must have been considerable. Most of the seed germinated vigorously in the plates without evidence of a fungus of any kind being present.

TABLE 1.—*Results obtained in 1932 from cultures made from the receptacles, cores and seed of tomatoes produced on vines severely diseased with Verticillium wilt*

Variety	No. of diseased plants	Total fruit cultured	Total receptacles infected with *Verticillium*	Total receptacles discolored	Total cores infected with *Verticillium*	Total cores discolored	Total no. of seed yielding *Verticillium*
Hybrids	14	287	44	40	7	71	0

Hundreds of the tiny plants were crushed or cut in half in the plates with sterile scissors and pressed deep into the agar to induce the fungus to establish colonies there if it were present in the vascular system. The fungus never appeared.

The experiment reported above quickly showed no relation to exist between the discoloration in the receptacle and the core; both may be discolored in the same fruit, or possibly the receptacle alone may show discoloration and the core none. And *vice versa*. It will be noted that there was vastly more discoloration observed in the cores than in the receptacles. This was probably due, in part at least, to the fact that the considerable size and whitish color of the circular discs of core tissue transferred to the plates made any discoloration present easy to detect, whereas the dark green and small size of the receptacles made it more difficult or uncertain. It was observed that discoloration became more pronounced in the sections of core tissue with passing time, possibly due to oxidation. Often neither receptacle nor core showing pronounced discoloration yielded the fungus in culture, which is in line with the observations of several workers who have noted that the discoloration of the vascular system of plants affected with Verticillium wilt may precede the actual invasion of the tissue by the fungus

TABLE 2.—*Results obtained in 1932 from cultures made from the seed of tomatoes, the receptacles of which were infected with Verticillium*

Variety	No. of diseased plants	Total receptacles infected with *Verticillium*	Total no. of seed cultured	Total no. of seed yielding *Verticillium*
Break O'Day	3	7	1202	0
Hybrid	5	5	1100	0

(4, 5). Occasionally, *Alternaria* sp. was isolated from the sections of discolored core tissue.

When it became apparent that no correlation existed between the discoloration of the receptacle and core, the plan of the experiment during the balance of the year was simplified; cores were no longer removed from the fruit, but cultures of the seed were made from all tomatoes, the receptacles of which had yielded *Verticillium* in culture. A count of the seed cultured also was made (Table 2).

TABLE 3.—*Results obtained in 1933 from cultures made from the receptacles, cores and seed of tomatoes produced on vines severely diseased with Verticillium wilt*

Variety	No. of diseased plants	Total fruit cultured	Total receptacles infected	Total cores infected	Total seed cultured	Total no. of seed diseased
Hybrids	10	64	18	1	1121[a]	1

[a] From 9 small fruits. Time would not permit of culturing the seed of the remaining 9 with infected receptacles.

Again, it was observed that much of the seed germinated normally. Neither the seed nor the young plants clipped in half or crushed yielded *Verticillium* when pressed deep in the agar.

Results Obtained in 1933. Cultures were made from the main stem, receptacle, core tissue, and the seed in the manner described (Table 3).

It will be observed that one seed cultured in 1933 yielded *Verticillium*.

Results Obtained in 1936. After a lapse of 3 years the work was resumed. Cultures were again made from the main stem, receptacle, and seed from those tomatoes, the receptacles of which had yielded *Verticillium* in culture. None of the seed cultured yielded *Verticillium* (Table 4).

TABLE 4.—*Results obtained in 1936 from cultures made from the receptacles and seed of tomatoes produced on vines severely diseased with Verticillium wilt*

Variety	No. of diseased plants	Total fruit cultured	Total receptacles infected	Total seed cultured	Total seed yielding *Verticillium*
Hybrids	15	41	3	193[a]	0

[a] From only 2 of the 3 fruits with diseased receptacles.

TABLE 5.—*Results obtained in 1940 from cultures of seed of tomatoes, the receptacles of which were infected with Verticillium. Also the total seed count of tomatoes produced on the same plants, the receptacles of which were not infected*

Variety	No. of plants infected with *Verticillium*	Total fruit studied	Total receptacles infected	Total seed cultured from fruit with infected receptacles	Total no. of diseased seed	Total seed count from fruit with healthy receptacles
Pearson	5	79	0	0	0	21,117
Essar	2	38	0	0	0	8,098
Early Santa Clara Canner	3	51	1	245	0	11,504
San Marzano	13	310	0	0	0	20,056
Break O'Day	31	457	3	465	0	84,916
Total	54	935	4	710	0	145,691

Results Obtained in 1940. After a lapse of 4 years the experiment was resumed on a very large scale made possible by the Work Projects Administration, which organization furnished laboratory technicians to assist with the work. Never less than 2 and as many as 4 men and women, trained by the writer to make cultures, were kept steadily on the job from the beginning of the harvest season until frost had destroyed the vines at the Deciduous Fruit Field Station. Then the work was continued with tomato plants and fruit brought from Salinas in Monterey County, where milder temperatures ordinarily prevail, until frost eventually destroyed the vines there, also. Cultures were made from the seed of all tomatoes, the receptacles of which had yielded *Verticillium* in culture. Counts were made not only of the seed cultured, but also of all other seed produced on the same plants by tomatoes, the receptacles of which had not yielded *Verticillium* in culture. Such seed necessarily must have been healthy. The counts were made simply to determine the total seed production of the diseased plants at the time they

TABLE 6.—*Results obtained in 1941 from cultures of seed from tomatoes, the receptacles of which were infected with Verticillium. Also, the total seed count of tomatoes produced on the same plants, the receptacles of which were not infected*

Variety	No. of plants infected with *Verticillium*	Total fruit studied	Total receptacles infected	Total seed cultured from fruit with infected receptacles	Total no. of diseased seed	Total seed count from fruit with healthy receptacles
Essar	1	17	0	0	0	2,126
Hybrid	4	56	1	210	0	14,389
Break O'Day	37	920	94	21,810	1	197,605
Essary[a]	21	472	4	422	0	68,284
Total	63	1,465	99	22,442	1	282,404

[a] The Essary tomato, definitely susceptible to *Verticillium* wilt, produced by the Tennessee Agricultural Experiment Station (1) is not to be confused with the Essar, a resistant variety produced by the University of California.

were dug. All plates were read under the microscope by the writer. The results are given in table 5. *Verticillium* did not develop in culture from any of the 710 seed.

Results Obtained in 1941. In this, the closing year of the experiment, exactly the same technique used in 1940 was employed. Again the cultures were made by assistants furnished by W.P.A. The results are given in table 6.

As seen in table 6, one seed among the 22,442 cultured yielded the fungus.

DISCUSSION

During the course of the experiments reported in this paper a minimum[5] of 26,768 seed from 180 tomatoes, the receptacles of which had been proved to be infected, were cultured, and of these only two seeds yielded *Verticillium;* one in 1933 (Table 3), and one in 1941 (Table 6). A total of 164 plants of 5 varieties and several hybrids representing extremes in resistance to the disease were used, including the Break O'Day (most susceptible), Essar (least susceptible), Pearson, Early Santa Clara Canner, Essary, and San Marzano, but most of the work was done with the Break O'Day, purposely raised for use in the studies because of its pronounced susceptibility.

Sight must not be lost of the fact that the 26,768 seeds cultured from 180 tomatoes during the entire experiment did not represent the total seed production of the plants from which they were taken. Cultures were actually made from 2,792 receptacles of tomatoes from the same plants, but when they were found to be healthy, it was considered useless to culture the seed. Thus, the seed of those tomatoes whose receptacles were infected, represented only a relatively small portion of the total seed production of the plants. To further illustrate: in 1941, 63 seriously diseased plants produced 99 tomatoes whose receptacles were infected, and therefore, might reasonably have been suspected of containing diseased seed. These tomatoes produced 22,442 seed, only 1 of which yielded *Verticillium* in culture. The other seeds were healthy in every way, judging by the rapidity and vigor with which the great bulk of them germinated in the plates. But these same plants had 1,465 tomatoes on them at the time they were dug, the receptacles of which were not infected, and toward which no doubt could possibly be cast on the health of the 282,404 seeds they contained; if the fungus had not reached the receptacles, it goes without saying it could not have infected the seed of the fruit borne on them. Therefore, out of a total of 304,846 seeds produced by the 63 seriously diseased plants at the time they were dug, only 1 single seed was involved. The ratio between the diseased and healthy seed is such that the factor of possible seed transmission of the disease is utterly negligible and of no economic importance.

All of the data cited above were compiled from experiments with seed produced by seriously diseased plants. Tomato seed is produced commer-

[5] A minimum figure, since no record was kept of the number of seed cultured in preliminary stages of the work.

cially from healthy plants rather than diseased as far as possible; consequently, the ratio in commercial seed between the healthy and diseased, might logically be expected to be vastly more extreme than that found in the experiments reported here.

There can be no doubt that in exceedingly rare instances the fungus can ascend the vascular threads as far as the seed, but whether on the two occasions reported in this paper it actually parasitized the seed is not known for the following reason. Each tomato seed is embedded in a gelatinous matrix through which runs the exceedingly fine, delicate, almost translucent vascular thread or funiculus that connects the seed with the placenta. Considerable lengths of this vascular thread, often exceeding that of the seeds themselves, remain in the jelly surrounding the seeds when they are removed from the tomato experimentally or in commercial extracting processes. Thus the fungus, in the two instances reported in this paper, actually may not have reached the seed at all, but rather have been confined to the short length of vascular thread adhering to it. If so, in the fermenting process by which tomato seed is prepared commercially for the trade, this gelatinous substance, together with the vascular elements contained in it, would slough off taking the parasite with it and leaving the seed as healthy as any other. Neither of the two seeds with which the fungus was associated in culture germinated, which suggests that they were parasitized and killed. It is barely possible that had the seeds been kept longer they might have germinated, but in the writer's opinion this is doubtful. *Verticillium* rapidly developed in the cultures and enveloped the seed in typical colonies.

Assuming the seeds actually to have been parasitized and killed, then they could not have produced plants the following spring and, accordingly, could constitute no menace. Then again, assuming the seed to have been parasitized without being killed, there is no assurance the fungus itself would survive the winter to attack the germinating plants the following spring.

Kadow (2) has reported experiments in which he found as much as 56 per cent of the tomato seed he worked with to yield *Verticillium* in culture and from which he concluded the disease to be seed-borne (2, 3). Why the results reported in this paper are at such great variance with his is hard to explain. The writer respectfully submits the suggestion, however, that final proof that the disease is seed-borne rests on germination studies with such seed in the spring following infection to determine whether (1) the seed is viable, and (2) any of the plants produced of such seed in sterilized soil develops Verticillium wilt. By such experiments alone can it be determined definitely that the use of infected seed constitutes a hazard.

In the light of the considerable data presented in this paper, tomato seed extracted from fruit produced by vines affected with Verticillium wilt must be regarded as safe to use. The question arises as to how the disease is disseminated. *Verticillium albo-atrum* and its related forms are known to attack over 200 widely unrelated plants including stone fruits, bush fruits,

pome fruits, truck crops, field crops, ornamentals and weeds. Once introduced into the soil through the medium of diseased cuttings, nursery stock, etc., the fungus may be expected to attack any of the other susceptible plants that may be present or even live saprophytically on plant debris, manure, etc. That the very unsanitary custom of disking under old tomato vines at the close of the season to increase the humus content of the soil is fraught with danger and provides an excellent means of increasing the fungus in the soil is seen in the following observation.

At the Deciduous Fruit Field Station an employee made the mistake of raking dead, artificially infected, tomato vines, that had been grown for the first time on the soil, over a wooden barrier on to adjoining healthy soil where he stacked them in piles and burned them. Then he disked the ashes into the soil. The writer examined the sites of the bonfires and determined that many fragments of the main stems and heavier branches had withstood burning and were either disked under completely or left partially protruding from the soil. The following spring Harold E. Thomas, a staff member conducting extensive strawberry investigations at the Station, planted a bed of strawberry plants in the hitherto healthy soil. Along the barrier, particularly in the spots where the tomato vines had been burned, and at no other place, the strawberry plants developed Verticillium wilt. The incident established beyond all doubt the ease with which the fungus may be introduced into healthy soil by unsanitary cultural practices. In this same way infective débris might easily be transported on farm machinery or implements from one piece of ground to another.

The writer does not wish to infer dissemination of the disease by the seed to be impossible, but rather, highly improbable. At least, such dispersal must be regarded as utterly insignificant and of no economic importance in comparison with the other means briefly referred to here.

SUMMARY

Experiments were conducted during five different seasons over a period of 10 years to determine whether tomato seed produced on vines severely attacked by Verticillium wilt might harbor the fungus. The receptacles of 2,792 tomatoes produced on 164 severely diseased plants were cultured but only 180 were found to be infected. More than 26,768 seeds, taken directly from these 180 tomatoes with diseased receptacles, were cultured. Only 2 seeds yielded the fungus. The plants used consisted of several hybrids and the following varieties: Pearson, Break O'Day (most susceptible), Essar (least susceptible), Essary, Early Santa Clara Canner, and San Marzano. The total seed cultured represented a relatively small number, only, of the total seed produced by these same plants, since most of the tomatoes were borne on receptacles which did not yield *Verticillium* in culture and, accordingly, must have been healthy.

The experiments revealed no significant relation between the discoloration of the vascular system in either the receptacle or the core; both may be dis-

colored in the same fruit, or possibly the receptacle may show discoloration, and the core none. And *vice versa*.

The great bulk of seed produced by diseased plants germinated readily and produced healthy plants in the culture plates. Large numbers of these plants were either crushed or cut in halves with sterile scissors and pressed back into the agar to enable the fungus, if present in the vascular system, to establish colonies. None developed.

The fact that two seeds yielded *Verticillium* in culture did not necessarily prove them to be infected, since in each instance the fungus may have been confined to the funiculus embedded in the gelatinous matrix that surrounds each tomato seed. If so, the ordinary fermentation process by which tomato seed is prepared for the trade would rid the seed of the gelatinous envelope, the funiculus, and the fungus contained in it. Neither of the two seeds germinated, which might indicate that they had been parasitized and killed. If so, they could have constituted no factor in the dissemination of the disease when planted. Assuming the seed to have been parasitized but not killed there is no assurance that the fungus would live over winter to attack the young seedlings the following spring.

The writer does not wish to infer that seed cannot become infected and actually transmit the disease but, rather, considers it to be highly improbable and certainly of no economic importance. Other means of dissemination are discussed.

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CHALARA QUERCINA N. SP., THE CAUSE OF OAK WILT[1]

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INTRODUCTION

Oak wilt is probably the most important disease of oak trees, particularly of *Quercus borealis* Michx. (red oak) and *Q. velutina* Lam. (black oak), in Wisconsin and neighboring States. It is characterized by wilting and death of the foliage, followed usually the same year by death of the tree. The symptoms of the disease and proof of the pathogenicity of the causal fungus have already been given (2). The description of the pathogen is the purpose of the present paper.

THE PATHOGEN

Macroscopic Cultural Characters

The macroscopic characters given, unless stated otherwise, are of 1-week-old cultures grown on 2 per cent malt agar[2] in Petri dishes at 25° C. The aerial mycelium was fluffy, 1–3 mm. high, and light gray (3, Plate 2, A 1) to olive-green (3, Plate 15, L 4) in color by reflected daylight. Occasionally present were raised patches of tan mycelium on which were droplets of clear to amber-colored liquid, which sometimes contained suspended conidia. The surface and sub-surface mycelium was hyaline to olive-green by transmitted daylight. Through the bottom of a Petri dish a culture appeared darkest toward the center with irregular areas and/or radiating lines of olive-green mycelium toward the hyaline margin. The margin was entire or nearly so. As the cultures aged, the tan color often became more predominant, the mycelium more appressed, and sclerotia often appeared. Loosely knit, irregularly shaped, tan to black sclerotial masses up to 2.5 cm. in diameter were observed in 3-month-old cultures in 6-oz. bottles. Cultures 1 to 3 weeks old had a characteristic odor suggesting old apple cider.

The diameter of 1-week-old cultures growing directly from chips of diseased wood varied from 1 to 2.5 cm. (Fig. 1, A). First transfers from such cultures were 1.8 to 3.2 cm. in diameter at a like age. The optimum temperature range for growth was 24–28° C. on corn-meal, nutrient-dextrose and 2 per cent malt agars.[3] Slight growth appeared in 2 weeks time at the extremes of 8° and 32° C.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Acknowledgment is given to the Wisconsin Conservation Department for cooperation and encouragement in these investigations.

[2] The malt agar consisted of 20 g. Trommer's malt extract, 18 g. shredded agar, and distilled water to make 1 liter.

[3] The corn-meal agar contained 60 g. yellow corn meal, 15 g. shredded agar, and distilled water to make 1 liter; the nutrient dextrose as given in Riker, A. J., and Riker, Regina S., Introduction to Research on Plant Diseases, 116 pp., John S. Swift Co., Inc., St. Louis, Chicago, New York, Indianapolis, 1936; the 2 per cent malt agar as given in footnote 2.

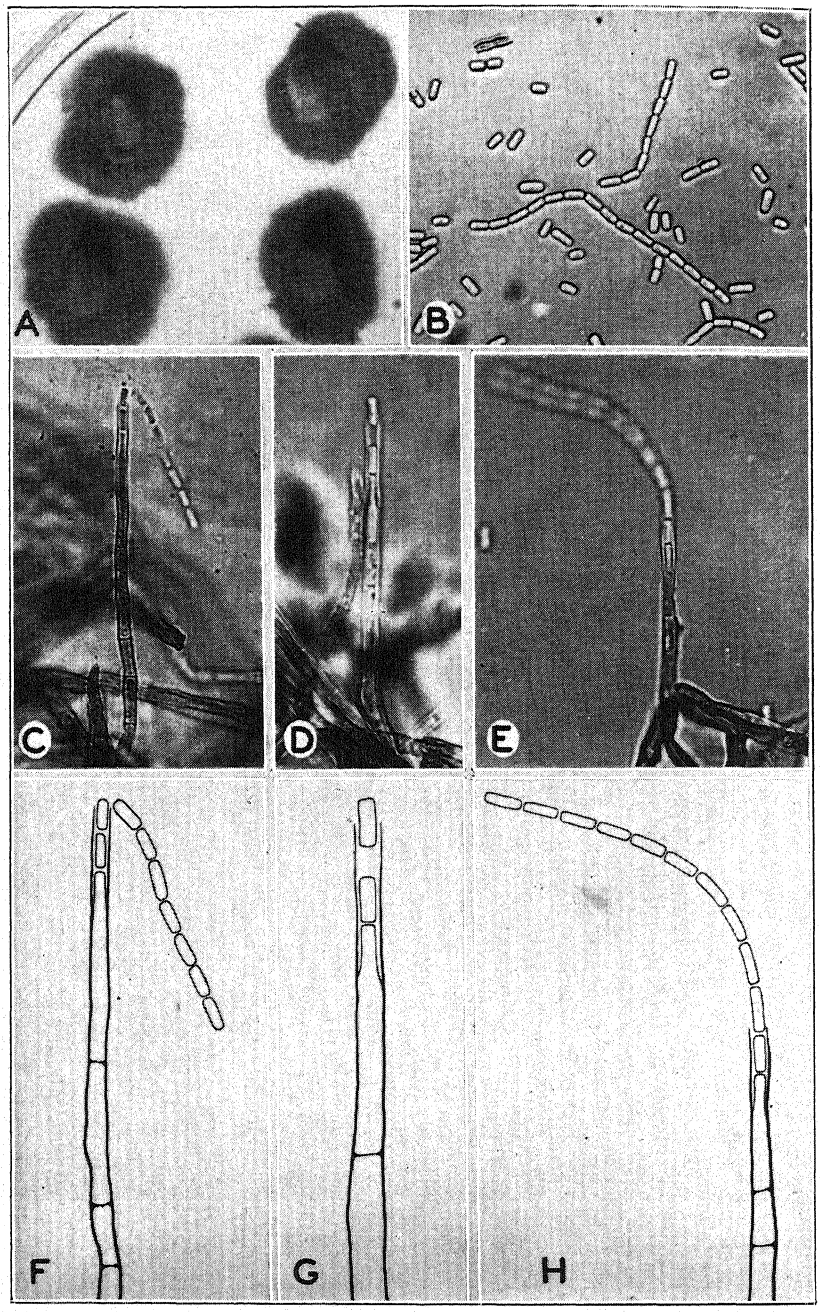


FIG. 1. *Chalara* **quercina** n. sp. A. Seven-day-old cultures from chips of diseased wood on 2 per cent malt agar in a Petri plate. B. Photomicrograph of conidia in a water mount. C, D, E. Photomicrographs of conidia and conidiophores. F, G, H. Silvertone drawings of C, D, and E, respectively. Measurements of colonies, conidiophores, and conidia are given in the text. Photographs and drawings by Eugene Herrling.

Few variations appeared in different isolates as they grew from chips of diseased wood or in the first transfer from these original isolations. In such cultures the fungus was readily identified by macroscopic characters. As the isolates were kept in stock at room temperature, however, and as masses of mycelium were transferred to fresh malt or nutrient-dextrose agar slants every 3 to 6 months, variations occurred in the color of the mycelium, in the rate of growth, and in the amount of sporulation. Over 100 isolates from a like number of naturally infected trees were carried in stock for various periods of time. Six of these isolates, obtained in 1941, were proved pathogenic in 1942. By April, 1943, after they had been in stock in 6-oz. bottles almost 2 years, they all differed from the "normal" type by having a white mycelium, faster growth rate, and very few conidia. They could no longer be identified by their macroscopic characters, though the method of sporulation and the spore characters were unchanged. The change in colony type did not occur in all isolates simultaneously, nor in like manner. Some changed to the "white" type after 2 or 3 transfers (6 months to a year) and others not until later. The "white" type sometimes appeared first as a sector in an otherwise "normal" colony, and sometimes a transplant from a "normal" colony would give rise directly to the "white" type. When the "white" appeared as a sector in a colony, transplants from the "normal" portion gave rise to either "normal" or "white" colonies. There has been no clear case of the "white" type reverting to the "normal." Pathogenicity was not necessarily lost by the change to "white" type, because the only "white" isolate used for inoculation produced disease symptoms and was reisolated without color change.

Microscopic Characters

The hyphae were subhyaline to brown, septate, branched, and 2.5–6 μ in diameter. Sporulation was usually abundant in cultures 2–3 weeks old. The conidiophores were subhyaline to brown, septate, simple or branched, 2.5–5 μ in diameter, and often tapering slightly toward the apex (Fig. 1, C–H). The conidia were hyaline, continuous, cylindrical, truncate at each end, 2–4.5 × 4–22 μ (mean 3 × 6.5 μ), endogenous and catenulate (Fig. 1, B–H). They were borne in the terminal cell of a conidiophore and in artificial culture nearly always adhered end to end to form long chains (Fig. 1, C, E, F, H). Eighty-six spores were counted in one chain and undoubtedly other chains contained more. In 3 cultures, spores were seen clustered about the apex of the conidiophore to form an irregular head; these were rare exceptions, however. Three cases of spores emerging from conidiophores were observed in a water mount. A spore would slowly (20 minutes to several hours) move outward until about half its length protruded, and then would suddenly pop out and lie free beyond the neck of the conidiophore. Spores that emerged in water did not often remain together to form chains.

Germination of the spores was observed in distilled water and in 1.25

per cent and 2.5 per cent malt solutions at 16, 20, 24, 28, and 32 degrees C. The highest germination was 93 per cent at 24° C. after 14 hours in the 1.25 per cent malt solution. The optimum temperature for germination in both solutions and the water after 14 hours was 24° C. Percentage of germination at all temperatures and time periods was lowest in the distilled water. The spores germinated by a single germ tube, usually arising at one end of the spore, or by two germ tubes, one from each end of the spore. If allowed to remain in the water or malt solutions, the germinated spores gave rise to secondary endogenous spores. The secondary spores often were borne soon after germination of the primary spores, with the germ tubes of the latter converted into conidiophores.

After 1–3 days in the water or malt solutions the germinated primary spores and many of the secondary spores were large (10–20 μ in diameter) globular bodies that no longer resembled the normal spore type. The "swollen" appearance of the secondary spores was not evident, however, until after their emergence from the conidiophores. This "swollen" type of spores was found a few times in cultures about 2 weeks old. In every case they were found at the lower edge of agar slants where the margin of the colony was in contact with water of condensation. Several of these "swollen" spores were individually isolated, transferred to malt agar, and in every case produced cultures that were macroscopically and microscopically like those from normal spores.

The oak-wilt pathogen has not been found sporulating in nature, but it has been grown aseptically on living and dead oak wood in the laboratory and there produced spores and hyphae of the same size and type as those formed on agar. Numerous attempts were made to induce the formation of a sexual stage, but all failed.

Taxonomy[4]

The oak-wilt pathogen apparently belongs in the genus *Chalara* Corda of the Dematiaceae. Four species, *C. affinis* Sacc. and Berl. (5), *C. fusidioides* Corda (*C. longior* Sacc.) (4), *C. heterospora* Sacc. (5), and *C. setosa* Hark. (1, 6), have been described with oak as the habitat. The first 3 species were described as inhabiting dead oak wood, while the last was inhabiting dead oak leaves. None were reported as pathogens. The 4 above species also differ, respectively, from the oak-wilt pathogen as follows: *C. affinis* has the conidiophores in fascicles and narrow spores (10–12 × 2 μ); *C. fusidioides* (*C. longior*) has distinctly flask-shape conidiophores, narrow hyphae (2.5–3 μ), and long, narrow spores (18–20 × 2 μ); *C. heterospora* has 1–3 septate conidia; and *C. setosa* has 1 septate conidia. Thus, on the basis of the brief descriptions available, the wilt organism does not seem to belong to any of the known species of *Chalara*. The name *Chalara* **quercina** n. sp. is, therefore, proposed.

[4] Acknowledgment is made to Dr. M. P. Backus, University of Wisconsin, and Mr. Ross W. Davidson, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, for assisting in the taxonomic work.

Technical Description[5]

Chalara **quercina** n. sp. Mycelial mat fluffy, 1–3 mm. high, white, becoming gray to olive-green with occasional patches of tan; sterile hyphae septate, branched, sub-hyaline to brown, 2.5–6 μ in diameter; sclerotia, sometimes present, tan to black, loosely knit, irregular in shape, up to 2.5 cm. in diameter; conidiophores not sharply differentiated from sterile hyphae; spore-bearing cells terminating long or short branches, sub-hyaline to brown, often tapering slightly toward the apex, 2.5–4.5 × 20–40 μ; conidia 2–4.5 × 4–22 μ, endogenous, hyaline, cylindrical, truncate at each end, continuous, catenulate.

Habitat: In roots, stems, branches, and leaves of living oak trees (*Quercus alba* L., *Q. borealis* Michx., *Q. coccinea* Muench., *Q. macrocarpa* Michx., and *Q. velutina* Lam.) in Illinois, Iowa, Minnesota, and Wisconsin, U. S. A.

Chalara **quercina** n. sp. Mycelium in culturis tegeticulam mollem 1–3 mm. altam formans, primo albam denique griseam vel olivae colore, quondoque passim brunneo-subflavam; hyphis sterilibus septatis, ramosis, sub-hyalinis vel brunneis, 2.5–6 μ crassis; sclertiis aliquando extantibus brunneo-subflavis vel atris, laxe formatis, forma incerta, ad 2.5 cm. latis; conidiophoris hyphas steriles simulantibus; cellulis quae sporas generant longorum vel brevium ramorum extremitatibus locatis, sub-hyalinis vel fuscis, saepe prope apicem tenuatis, 2.5–4.5 × 20–40 μ; conidiis 2–4.5 × 4–22 μ, ex cellularum fertilium apice exsilientibus, hyalinis, cylindraceis, utrinque truncatis, continuis, catenulatis.

Habitat in radicibus, caudice, ramis, et foliis arborum vivarum quercinarum (*Quercus alba* L., *Q. borealis* Michx., *Q. coccinea* Muench., *Q. macrocarpa* Michx., and *Q. velutina* Lam.) in Illinois, Iowa, Minnesota, and Wisconsin, U. S. A.

Type material has been filed in the Cryptogamic Herbarium of the Department of Botany, University of Wisconsin, Madison, Wisconsin, and in the "Mycological Collections," Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

SUMMARY

The causal fungus of the oak wilt that attacks *Quercus alba, Q. borealis, Q. coccinea, Q. macrocarpa,* and *Q. velutina* in Illinois, Iowa, Minnesota, and Wisconsin is herein described and the name *Chalara* **quercina** n. sp. is proposed.

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[5] Latin translation by Dr. M. P. Backus, University of Wisconsin.

OAK WILT: ITS SIGNIFICANCE, SYMPTOMS, AND CAUSE[1, 2]

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INTRODUCTION

An extensive dying of oak trees in Wisconsin and the Upper Mississippi Valley has aroused concern for some years. In 1881 Warder (5) discussed dying black oaks in Madison, Wisconsin. A number of notes made in 1912 by Hartley,[3] now filed in the Host Index, Division of Forest Pathology, U. S. Plant Industry Station, Beltsville, Md., recorded a dying of oaks, observed also by other pathologists both in Wisconsin and Minnesota. Various reports have been made by Tiemann (4), Lorenz and Christensen,[4] and others. Tiemann's paper alone gives an adequate description of the symptoms observed. Those he listed are in general agreement with the detailed description herein recorded.

The present paper presents evidence on the significance, symptoms, and cause of a disease called oak wilt, about which preliminary statements already have appeared (3, 7). The reasons for selecting the name oak wilt are given after a description of the symptoms. Although considerations of the relationships among various important items in the whole oak disease complex need further investigation, the present study has demonstrated one cause for the dying of oaks, particularly in the red oak group.

HOSTS

Although some of the species within the red oak group are difficult to distinguish because of intergrading characters, what appeared to be the red (*Quercus borealis* Michx. f.) and black (*Q. velutina* Lam.) oaks have been most seriously affected. Two wilting scarlet oaks (*Q. coccinea* Muench.) were found. Trees in the white oak group (*Q. alba* L. and *Q. macrocarpa* Michx.) also were attacked, but seemed relatively tolerant to the disease. This disease is important primarily on the red oak group.

ECONOMIC IMPORTANCE OF BOTH HOST AND DISEASE

The economic importance of oak trees has been estimated only in an indi-

[1] In cooperation with the Forest Products Laboratory, Forest Service, United States Department of Agriculture. During the early stages, the work on oak wilt was considerably advanced by the records taken by R. H. Gruenhagen, L. J. Meuli, and L. F. Roth.

[2] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

Assistance for some of this work was furnished by the personnel of the Work Projects Administration, official project No. 65-1-53-2349.

The photographs were made by Eugene Herrling except for figures 1, D, and 2, D.

[3] Carl Hartley, principal pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[4] Lorenz, Rolland C., and Christensen, Clyde M. A survey of forest tree diseases and their relation to stand improvement in the lake and central states. U. S. Bur. Plant Indus. 52 pp. illus. 1937. [Processed.]

rect manner. According to farm census figures provided by Walter Ebling,[5] the annual income from all species in farmers' woodlands has varied according to prevailing prices for 1929 and 1939, respectively, between about 17 and 10 million dollars. This income may be divided at approximately 70 per cent used on the farm and 30 per cent sold off the farm. Within this total income much (5½ million dollars in 1937) was derived from oak trees, according to a survey by the Forest Service and the Bureau of Agricultural Economics. These figures have not included trees in municipal, county, State, or federal parks, or on private lawns, where the aesthetic value of trees is difficult to estimate. In southern, southwestern, and western Wisconsin, oaks comprise about 80 per cent of the trees in woodland. They are among the natural resources not easily and quickly replaced. Although a more exact figure cannot be given for the economic importance of oak trees threatened by wilt, their value is obviously great in Wisconsin alone.

The damage caused by oak wilt depends on several considerations. According to F. B. Trenk:[6] (1) The full value of immature trees killed by wilt cannot be realized by any salvage operations. (2) After such oaks have died, a number of years, when the land may be considered "idle," are necessary until replacement trees of other species can grow to an equivalent size. (3) For certain locations the native oak trees may be better adapted for growth than other species. Such loss caused directly by oak wilt is common and serious in at least 30 Wisconsin counties. A similar view has been expressed by C. L. Harrington.[7]

A lawn or park tree killed by wilt must be taken away. Thus to the aesthetic loss is added a removal cost.

Much of the reproduction on oak lands is by the growth of sprouts from old stumps. This is prevented in wilt-infected areas. Although the roots and root collars of wilted trees may remain alive for some time, they usually harbor the pathogen. Thus, they either fail to sprout or produce sprouts that soon wilt.

The incidence of the disease has been irregular in both mature and immature stands. An example of high incidence in an immature stand is given under "Distribution and Spread." On the other hand, many areas within the boundaries of the known geographical distribution have been free from the disease at least for the past 2 years.

Oak wilt is probably the most important of the various diseases (1, 2) of red and black oaks in the Upper Mississippi Valley. However, more specific data on the economic importance cannot be given until detailed surveys have been made.

DISTRIBUTION AND SPREAD

The authors have observed the disease as widespread in southern, southwestern, and western Wisconsin. The wilt fungus has been isolated from wilting trees in 23 counties in Wisconsin, 5 in Minnesota, 2 in Iowa, and

[5] Division of Agricultural Statistics, Wisconsin Department of Agriculture.
[6] Extension Forester, Wisconsin Agricultural Experiment Station.
[7] Superintendent of Parks and Forests, Wisconsin Conservation Department.

1 in Illinois.[8] The distribution was examined in relation to local conditions.

The kind of soil in which the trees were growing was considered in relation to the distribution of oak wilt. Soil samples were collected near the base of wilting trees in 31 localities in Wisconsin in 1941 and 1942. These were analyzed by H. H. Hull[9] to determine whether any correlation could be established between the kind of soil, the nutrients available, the acidity, and the development of wilt. Wilted trees were found on the following soil types: fine sand (*e.g.,* Plainfield sand), light- and medium-colored sandy loam, light- and medium-colored fine sandy loam, light- and medium-colored silt loam, and light- and medium-colored loam. The available phosphate in the top soil ranged from 10 to 400 pounds per acre, and that in the soil 8 to 12 inches deep, from 5 to 200 pounds. The available potassium in the top soil ranged from 60 to 600 pounds per acre, and that in the soil 8 to 12 inches deep, from 60 to 160 pounds. The acidity of the top soil ranged from pH 4.5 to 8.0, and that of the soil 8 to 12 inches deep, from pH 4.8 to 7.5. Since no correlation was found between the kind of soil, the nutrients present, or the soil reaction and the incidence of oak wilt, the development of wilt appeared to be independent of such factors.

The relation of site to the incidence of oak wilt was examined. Since trees with wilt were found near swampy land, on hillsides and plateaux, in open fields, in woods with closed canopies, in pastured and nonpastured woodland, and, since these included good, indifferent, and poor situations for growth, the occurrence of wilt appeared not to be distributed according to site.

The spread of the disease in a woodland has fortunately been slow. Commonly a tree at the edge of a woodland first showed the disease. From this point the disease seemed to spread in a more or less circular manner, a few to several trees dying each year. During the growing season such an area has presented a striking picture with the point of initial infection surrounded by dead and dying trees. As an extreme example, an area of about 4 acres of sprouts and seedlings from 1 to 4 inches d.b.h., observed in 1942 in eastern Minnesota, included an estimated 200 dead trees and 75 wilting ones.

In nature, where one or more sprouts in a group wilted, the other sprouts from the same stump also wilted in the same or the following year. Similar results have been observed in 7 cases following artificial inoculations. In such instances the spread was probably through the living vascular tissues of the old stump that served as a direct connection between the sprouts. The causal fungus has been isolated from such connective tissues.

Since anastomosis of roots of oak trees does occur underground, as described by Weir (6) for black oaks and as shown in figure 1, D, for white oaks, this may be one means of spread from tree to tree. However, the separation of localized diseased areas by a distance of several hundred yards

[8] Acknowledgment is made to Messrs. C. M. Christensen, R. K. Alman, and J. C. Carter for aid in these studies in the respective neighboring States.

[9] Instructor in Soils, University of Wisconsin.

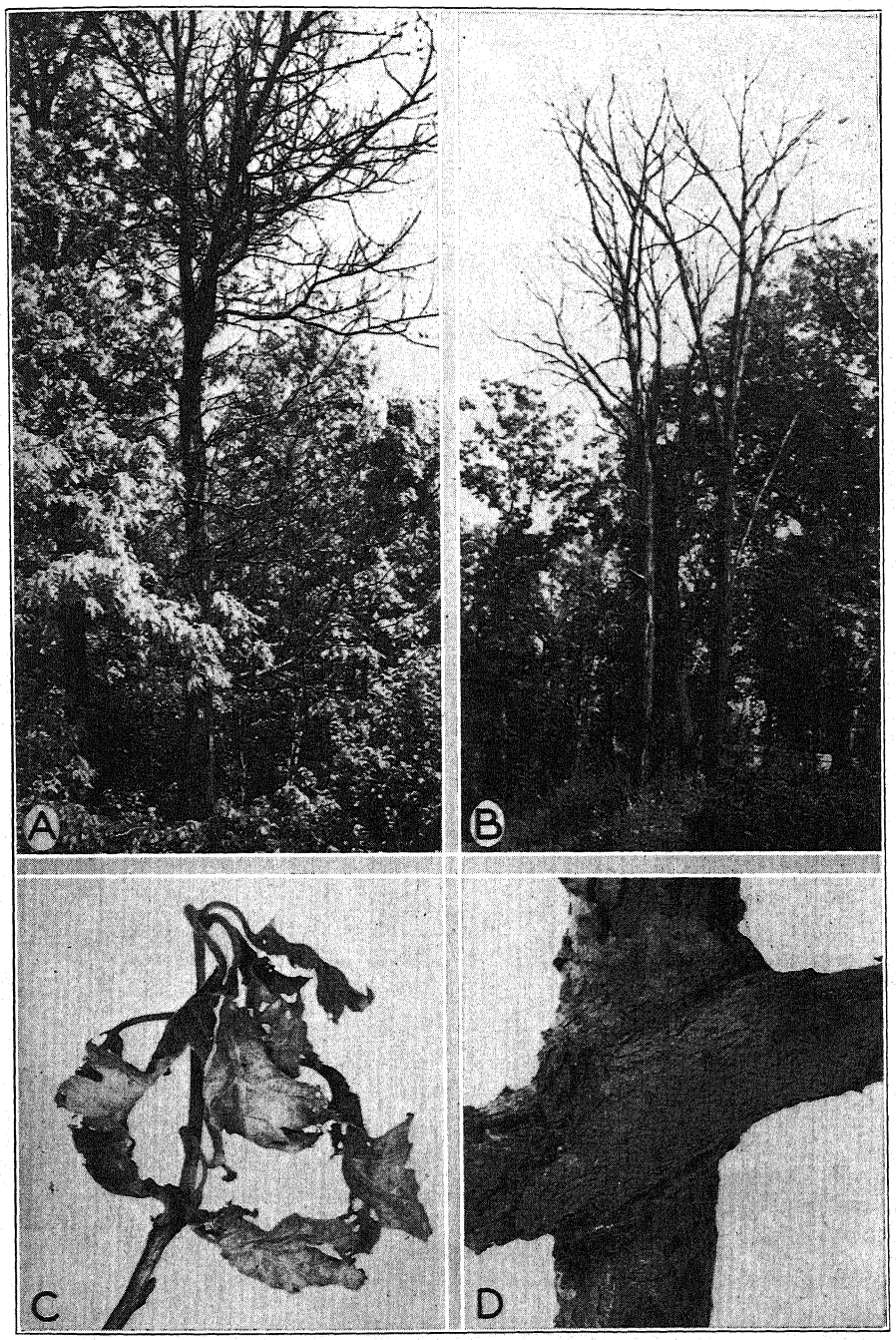


FIG. 1. A, Black oak with a 10-inch d.b.h., which died following inoculation. B, A group of oak trees that died in nature, with symptoms of oak wilt. C, Twig from inoculated black oak, showing wilted young leaves. D, Roots of white oak, showing anastomosis.

to a mile or more, and the wilting of an occasional tree in an area where there had been no previous sign of wilt, indicated that there were also other means of spread.

SYMPTOMS

The symptoms given below were observed during the summers of 1941 and 1942 on diseased trees from which the causal organism was isolated. Trees in the first stages of the disease were found from late May throughout the summer until late September, when natural autumnal coloration of the leaves interrupted symptom expression. However, the disease became conspicuous in most cases in June and July. The size of oaks known to have wilt ranged from 1 to 30 inches in diameter at breast height.

On Red Oak Group

The first macroscopic symptoms on mature leaves were slight crinkling and paling of the leaves, usually near the top of the tree or toward the tips of lateral branches. Affected leaves often became bronze to brown progressively from the apex and tips of the lateral lobes toward the base of the leaf blade. The last part of bronzing leaves to remain green was the blade tissue adjacent to the petiole. In some cases the base of the petiole became black. The mature leaves remained relatively stiff during the various stages and after death.

Young leaves, such as those appearing in May, usually did not show the bronze to brown color. As they wilted they became nearly black progressively from the tip of the blade toward the base and were curled and drooped (Fig. 1, C). They remained attached after wilting.

Affected mature leaves fell at any of the symptom stages; thus it was often possible to find fallen leaves showing various stages from pale green through progressive phases of bronzing and browning. Defoliation was slight to nearly complete. A diseased tree was often conspicuous from a distance because of its discolored leaves.

The symptoms progressed very rapidly over the entire tree, commonly affecting the lower branches last. Wilting was usually complete within a few weeks. A unilateral development of symptoms was seldom observed.

As wilting of the primary leaves became complete, sucker growth to a varying degree often appeared along the main stem and larger branches. Leaves of this new growth in turn wilted and died as described for young leaves.

Brown to black discoloration often was found in the sapwood (Fig. 2, D) of diseased trees, usually in the most recent ring. Peeling back the bark revealed diffuse discoloration or stippled to continuous streaks of varying lengths (Fig. 2, B). This was not a dependable diagnostic character, however, because it was not always evident in wood from which the causal organism was isolated and could not always be differentiated from discoloration caused by other agencies.

The inner bark and wood died after wilting of the leaves. The small twigs died first, then the branches, the trunk, and finally the roots. The

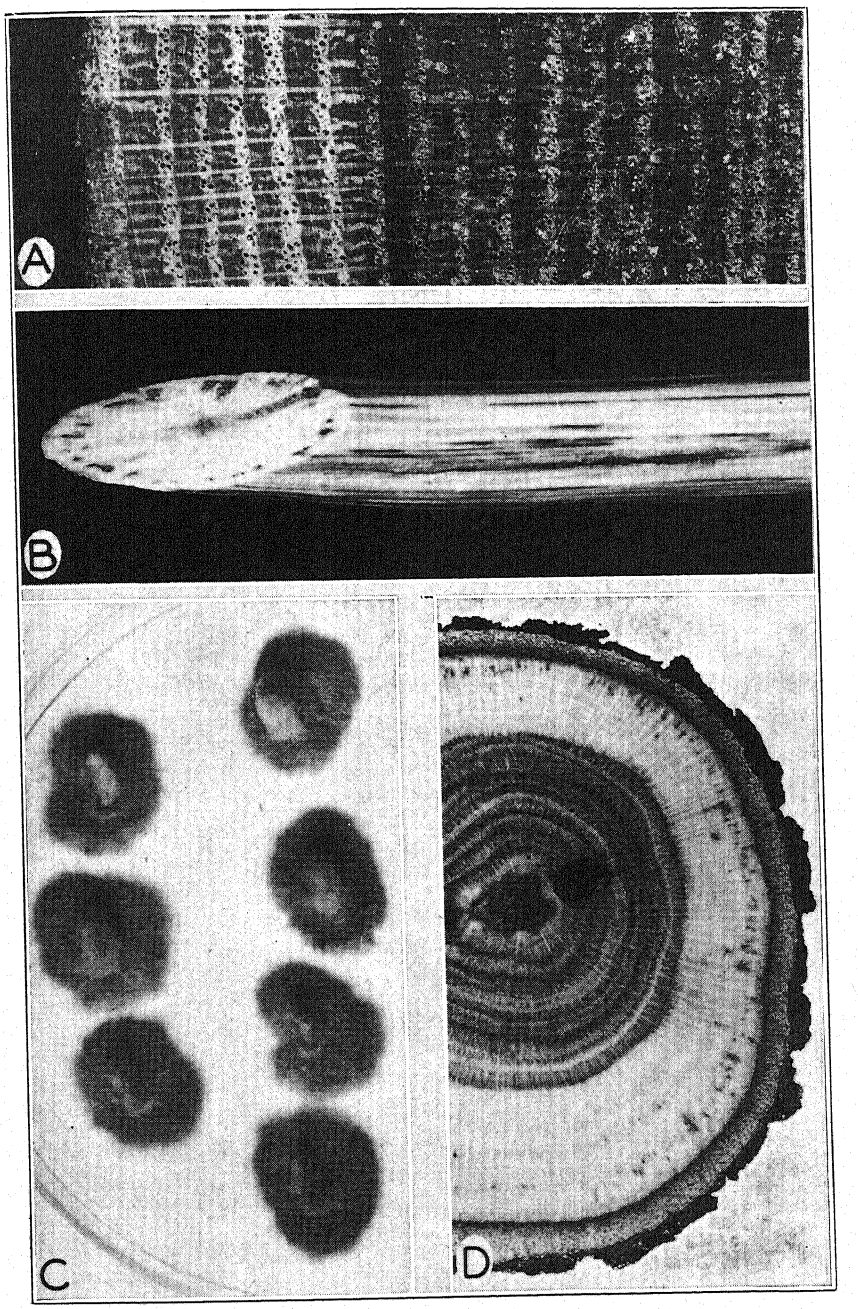


FIG. 2. A, A section of a killed tree. The most recent rings (left) show normal growth until the time of death. B, An infected black oak twig with bark removed, showing the discoloration that often appears. C, Growth of the oak wilt fungus from chips of diseased wood after 7 days at room temperature on malt agar. D, Cross section of a black oak stem photographed July 8, 1942, showing discoloration in the outer rings. The tree was inoculated August 27, 1941, was wilted September 30, made slight growth the spring of 1942, but died before the leaves were full size.

annual rings showed average growth up to the time of wilting. Such a situation was reported by Tiemann (4), and his illustration of it appears in figure 2, A.

Trees usually died during the summer that they first showed external symptoms, but in a few cases the larger branches lived over winter, partly leafed out the following spring, and then died. No tree of the red oak group has been known to recover.

Trees that died in nature with symptoms of wilt (Fig. 1, A) and a tree that died following inoculation (Fig. 1, B), as described later, are illustrated.

On White Oak Group

The symptoms on white and bur oaks differed in some respects from those on trees of the red oak group. Comparatively few trees of the white oak group were found diseased, and they were not so severely affected as the red oaks.

The leaf blades often became progressively necrotic from the apex toward the base, as described for red oaks. Though sometimes bronze, the discolored areas were often light tan to brown, or dark green and somewhat "water-soaked" in appearance. The withered, necrotic leaves tended to remain on the trees, so that leaf fall was seldom heavy.

Usually, in this host group, an entire tree did not wilt. Twigs with dead and dying leaves were often scattered throughout the crown, and some branches showed leaf symptoms while others remained outwardly healthy throughout the season.

Discoloration in the sapwood was found in each of the 14 trees examined, indicating that this might be a more common symptom in the white than in the red oak group. Most of the trees also showed dieback of the branches to varying degrees—perhaps a symptom associated with the comparatively slow progress of the disease on this host group.

The symptoms on both the red and white oak groups, though variable, allowed reasonably certain field identification. This was shown by the isolation of the causal fungus from 95 per cent of the trees suspected of having wilt.

Oak wilt was selected as a common name since it met the situation better than any other common designation available for the following reasons: (1) The progressive bronzing and browning from the margins on structurally stiff mature leaves suggested insufficient moisture. (2) Affected young leaves were curled and drooped. (3) There was often discoloration in the outer layers of sapwood. (4) The causal fungus was present in the vascular system. (5) This name has been used in Wisconsin for several years.

THE CAUSAL ORGANISM

Methods of Isolation

The method[10] of isolation from woody tissues was as follows: The diseased specimens generally used were twigs ¼ to 1 inch in diameter and about

[10] A similar method was in common use at the Forest Pathology Laboratory, Morristown, New Jersey.

6 inches long, taken from a wilting branch. About half the length of a section was dipped into a jar of 95 per cent alcohol, passed through a flame, and the alcohol allowed to burn. The bark was peeled or shaved from the flamed portion with a knife sterilized in like manner. After second sterilization of the knife, radial chips 1 to 3 mm. wide and deep enough to include at least the 2 outer growth rings were cut in the exposed wood, removed with sterile forceps, and set into the medium in a Petri dish. A satisfactory medium consisted of 1.5 per cent agar and 2 per cent Trommer's malt extract. Ten chips cut in linear succession from a twig sample were placed in a single dish. Isolations were made from at least 4 samples from an individual tree, making a total of 4 plates and 40 chips. The plates were incubated at about 25° C., and the causal fungus usually made sufficient growth in 7 days to be identified by macroscopic characters (Fig. 2, C). Similar techniques were used in isolating from trunk and root samples.

A few isolations were made from leaves of diseased trees in studying the distribution of the wilt fungus in the host. Isolations generally were attempted only from leaves that showed the progressive bronzing and were not yet dried out. The leaves were washed for 10 to 15 minutes in a 1 to 10 dilution of 4.6 per cent sodium hypochlorite solution and then in distilled water. Sections about 5 mm. in length and of desired width were cut with a sterile knife on a board soaked in the sodium hypochlorite dilution. The sections were then inserted directly into the medium in the Petri dish. Sometimes, with equally satisfactory results, the sections were washed also in 70 per cent alcohol and twice in sterile distilled water before being put into the dishes. The same medium and temperature were used as those described for the wood isolations. Because of slower fungus growth from the leaf sections, the plates were kept from 10 to 14 days.

Results from Isolations

During the summers of 1941 and 1942 samples were taken from 122 oak trees suspected of having the wilt (Table 1). Of these, 108 were of the red oak group and 14 of the white oak group. The causal fungus was isolated from samples of 104 of the 108 trees in the red oak group and from 12 of the 14 trees in the white oak group, or 95 per cent of the suspect trees. Of the 116 positive cases, 106 were determined by branch or twig isolations, 6 by trunk isolations, 2 by branch and trunk isolations, and 2 by branch, trunk, and root isolations. Isolation attempts were nearly always successful from diseased wood that was still green but rarely successful from wood that was dead or nearly so. Secondary fungi, especially *Dothiorella quercina* (Cke. and Ell.) Sacc., usually overran the plates in the latter case.

In 1941, samples also were taken from 56 oak trees not showing typical wilt injury, *i.e.*, nonwilt trees (Table 1), to determine whether other symptoms were caused by the oak-wilt pathogen. Some of these trees showed dieback, staghead, twig blight, cankers, and other difficulties, but none yielded the oak-wilt fungus.

Among the few leaf isolations attempted, only a small percentage was successful. The causal fungus was isolated from a total of 8 leaves collected from 3 naturally wilting trees. The fungus was isolated also from the wood of these trees. Six of the leaves were picked from the ground and 2 from a wilting branch. The fungus was isolated from the petioles of all 8 leaves, from the midribs or larger veins of 6, and from the green portions of the blades of 2. Several attempts were made to isolate the pathogen from the bronzed portions of the leaf blades, but all failed.

TABLE 1.—*Results of isolations from naturally wilted and nonwilted oak trees*

Species	Studies of 1941		Studies of 1942	
	Trees used for isolations	Trees yielding wilt fungus	Trees used for isolations	Trees yielding wilt fungus
	No.	*No.*	*No.*	*No.*
Wilt trees:				
Quercus borealis	18	17	39	38
Q. velutina	21	21	28	26
Q. coccinea	1	1	1	1
Q. alba	1	1	4	3
Q. macrocarpa	1	1	8	7
Total	42	41	80	75
Nonwilt trees:				
Q. borealis	19	0	.....	.....
Q. velutina	13	0	.....	.....
Q. alba	19	0	.....	.....
Q. macrocarpa	5	0	.....	.....
Total	56	0	.....	.....

Growth Characters of the Fungus

The oak wilt seemed continually associated with an undescribed parasitic fungus inhabiting the vascular tissues of the host. The mycelium of cultures from diseased wood was rather fluffy, and gray to olivaceous green. Cultures 1 to 3 weeks old had a characteristic, fermenting odor, somewhat reminiscent of old apple cider. Only asexual sporulation of the fungus has been observed. Conidia were abundant on 2-week-old malt agar cultures grown at 20° to 25° C. and were cylindrical, endogenous, and catenulate.

The fungus apparently belongs among the *Dematiaceae* and probably in the genus *Chalara* Corda. A more complete description and a classification are being published by Henry.

Methods of Inoculation

The field plot selected for inoculations was an ungrazed aspen and oak woodland near Madison, Wis. There was no oak wilt within ¼ mile.

One isolate, 1 mixture of 2 other isolates, and 6 single-spore cultures of the fungus were used for inoculations. The isolates were obtained during the summer of 1941 from naturally infected trees and represented 8 different localities in southern Wisconsin (Table 2).

The cultures used for inoculations were grown at room temperature for about 2 weeks on sterilized corn meal or rice in Petri dishes.

Inoculations were made in August, 1941, and in June, July, and August, 1942.

Trees selected for inoculations were mostly black oaks of sprout origin and from 1½ to 5 inches d.b.h. Exceptions were one 8-inch and one 10-inch d.b.h. black oak, one 2½-inch and one 6-inch white oak inoculated in 1941, and one 2½-inch white oak inoculated in 1942. All trees were in good health when the experiments were started.

TABLE 2.—*Results of inoculating oak trees with the wilt fungus*

Fungus isolates			Experimental trees[a]		
Serial letter[b]	Origin		Inoculated	Wilted	Wilt fungus reisolated
	Kind of oak	Nearest Wisconsin town			
			No.	*No.*	*No.*
1941 trials:					
A	Black	Madison	32	27	27
B	Red Red	Baraboo Prairie du Chien	5	4	4
Totals			37	31	31
Controls			21[c]	0	0
1942 trials:					
A	Black	Madison	19	16	16
C	Black	Waupaca	12	12	12
D	Red	Baraboo	4	3	3
E	Bur	Verona	4	4	4
F	Red	Lake Geneva	4	4	4
G	Scarlet	Lake Ripley	4	4	4
H	Black	Madison	6	0	0
Totals			53	43	43
Controls			8[d]	0	0

[a] All were black oaks except 3, which were white; 2 inoculated with isolate A in 1941, and 1 inoculated with isolate H in 1942, as explained in the text.

[b] A was a mass isolate and B a mixture of 2 mass isolates. C, D, E, F, and G were single-spore cultures. H was a single-spore culture from isolate A.

[c] Twelve wounded but not inoculated and 9 unwounded.

[d] Wounded but not inoculated.

Inoculations were made in the stems at 2 to 6 feet above the ground. Various methods were used in 1941, but all involved wounding the trees so that the mycelium and spores of the fungus were introduced into at least the 2 outer growth rings. Since all methods were about equally successful, only the chisel-cut method, which was the only one used in 1942, is described.

With the chisel-cut method of inoculation the rough outer bark was smoothed off from about 6 square inches, the pared surface was washed with 95 per cent alcohol, and it was allowed to dry. A ½-inch wood chisel was dipped into 95 per cent alcohol, flamed, and then driven through the inner bark and the 2 outer rings of wood. The chisel was removed, and the resulting hole in the tree was filled with mycelium and spores from a culture of the wilt fungus. To prevent too rapid drying of the inoculum, a wad of absor-

bent cotton was soaked in sterile water and bound over the wound with a strip of unbleached muslin. In 1941, from 1 to 6 inoculations, depending on tree size, were made around each tree in spiral fashion. In 1942, the number was reduced to 1 in most cases, with only a slight drop in percentage of infection. Control trees were either untreated or they received treatment like that given the inoculated trees, except that sterile medium was placed in the wounds.

Results from Inoculations

The inoculation results have been recorded in table 2. The first appearance of symptoms in the 1941 trials was noted 27 days after the August inoculations. By the end of September, when natural autumnal coloration of the leaves began to interfere with foliar symptoms, 19 of the 37 inoculated trees had developed leaf symptoms. Nine additional trees wilted in May, 1942, before the new leaves reached maturity. In June, 1942, 3 more trees wilted after having leafed out in apparently normal fashion. Thus a total of 31, or 84 per cent, of the 37 trees inoculated in August, 1941, developed foliar symptoms by the spring of 1942. The 2 white oaks and the inoculated 10-inch black oak were among the first trees to show symptoms. Both white oaks, however, seemed to recover almost completely during the summer of 1942, except for slight dieback on some of the small branches near the top. The foliage seemed normal, and the fungus was reisolated from only 1 of the trees in 2 attempts during the summer. None of the wilted black oaks recovered. However, a few that had wilted in the fall of 1941 started to leaf out the following spring and then wilted again before the leaves were matured.

In the 1942 inoculation series, the first appearance of symptoms was noted 18 days after the June inoculations. By the 25th of September, 43, or 81 per cent, of the 53 inoculated trees had developed leaf symptoms. The single white oak used did not become diseased. It was inoculated, however, with isolate H, which gave negative results throughout 1942. The symptoms on inoculated trees were comparable to those described for naturally infected trees. None of the control trees developed wilt symptoms.

Reisolation of the Original Fungus

Reisolations were usually made from lateral branches several feet from the nearest inoculation point soon after symptoms appeared. The original fungus was reisolated by the middle of November, 1941, from 28 of the 37 trees inoculated in August, 1941. These 28 positive cases included the 19 trees that showed symptoms by the end of September, 1941, and the 9 trees that first wilted in May, 1942. The fungus also was isolated at the time symptoms were noted from the 3 1941-series trees that did not wilt until June, 1942. The pathogen was reisolated from all 1942-inoculated trees that developed leaf symptoms. Repeated attempts to isolate from the 1941- and 1942-inoculated trees that did not develop symptoms, as well as from all control trees, have failed to yield the causal fungus. The results are summarized in table 2.

Recovery of the original fungus from the roots, trunks, and leaves of some of the inoculated trees showed the distribution of the pathogen to be similar to that in naturally infected trees as discussed earlier.

SUMMARY

A destructive disease of oak trees, termed oak wilt, was studied in Wisconsin and neighboring States.

The disease has appeared widely distributed, and its presence has been confirmed, by positive cultural studies, in 23 counties in Wisconsin, 5 in Minnesota, 2 in Iowa, and 1 in Illinois. No correlation has been found between site and occurrence of the disease.

Wilt appears to be the most important oak disease in the Upper Mississippi Valley.

Red and black oaks were the most common hosts and have not been known to recover once they were attacked by the wilt disease. Two wilting scarlet oaks were found. White and bur oaks also were attacked, but seemed relatively tolerant to the disease.

The first symptoms were slight crinkling and paling of the leaves, often followed by a progressive bronzing to browning of the leaf blade from the lateral edges and apex toward the midrib and base. Mature leaves remained relatively stiff, but young leaves drooped conspicuously. Defoliation varied in extent and seemingly occurred at any symptom stage on mature leaves. Affected young leaves remained attached after wilting. Symptoms usually progressed over the entire tree within a few weeks. Secondary foliage often appeared along the trunk and larger branches, but this in turn wilted and died. Discoloration in the sapwood of twigs was found in numerous cases but was not a diagnostic character.

A fungus, tentatively placed in the genus *Chalara,* was isolated from 116 of 122 wilt trees sampled, but was not obtained from any of 56 nonwilt trees.

Stem inoculations with the fungus were successful on 74 of 90 woodland trees. Cultures from 8 different locations in Wisconsin were used. The fungus was reisolated from the 74 positive cases of wilt and was identified as that used for the inoculations.

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WEB BLIGHT OF SEEDLING TUNG TREES TENTATIVELY IDENTIFIED AS THE RHIZOCTONIA STAGE OF CORTICIUM MICROSCLEROTIA

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(Accepted for publication December 30, 1943)

Lower leaves of seedling tung trees covered with mycelium were observed by Samuel Merrill, Jr., in a tung nursery in Mississippi in June, 1942. Later, the same disease was found in a tung nursery in Louisiana, and has been observed again in tung nurseries in 1943. This disease usually is found on leaves 6 to 18 inches above ground, and causes them to turn brown and die.

Young infection spots are light-tan and 0.2–2.0 cm. in diameter (Fig. 1, A). As the lesions grow larger, light-tan hyphae develop on the shaded side of the leaf. These hyphae extend rapidly over the surface of the leaf blade, progressively killing it (Fig. 1, B). Under conditions of high humidity the hyphae spread out and cover both the blade and the petiole. Often the hyphae grow to adjacent leaves, causing them to stick together (Fig. 1, D). Old diseased leaves have a very ragged appearance (Fig. 1, C). Often numerous grains of sand stick to the hyphae. During a rainy period the petiole and blade of an infected leaf may be covered with fine, close-growing, entangled hyphae that bear clumps of light-brown sclerotia (Fig. 1, C).

Diseased tissues cultured on water-agar and on Lima-bean-agar plates developed distinct Rhizoctonia features. Microscopic measurements of 100 sclerotia, found on the upper part of the petioles of infected leaves, ranged from 80–200 × 80–300 μ, averaging 150 × 200 μ. These measurements are within the range of those for *Corticium microsclerotia* (Matz) Weber, namely, 80–300 × 80–600 μ.[2] Bean plants inoculated with sclerotia from diseased tung leaves developed characteristic web blight, whereas check plants remained healthy and vigorous. Minute lesions appeared within 24 hours, and under controlled conditions sclerotia were observed three days after inoculation. Complete collapse of the bean leaves and defoliation occurred as the disease developed. Sclerotia from artificially inoculated bean plants and from naturally diseased tung plants, plated on water agar, developed cultures indistinguishable from each other.

The small size of the sclerotia, the growth of the fungus in water-agar cultures, and the general similarity of the symptoms on diseased tung trees to those described for web blight of beans suggest that this is the Rhizoctonia stage of web blight, *Corticium microsclerotia*. L. H. Persons, of the Emergency Plant Disease Prevention Project, inspected the nursery in September, 1943, and confirmed this diagnosis.

[1] Assistant Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Weber, G. F. Web blight, a disease of beans caused by *Corticium microsclerotia*. Phytopath. 29: 559–575. 1939.

The disease apparently is of minor importance to the tung nursery; but, under favorable environmental conditions, it is possible that a severe epi-

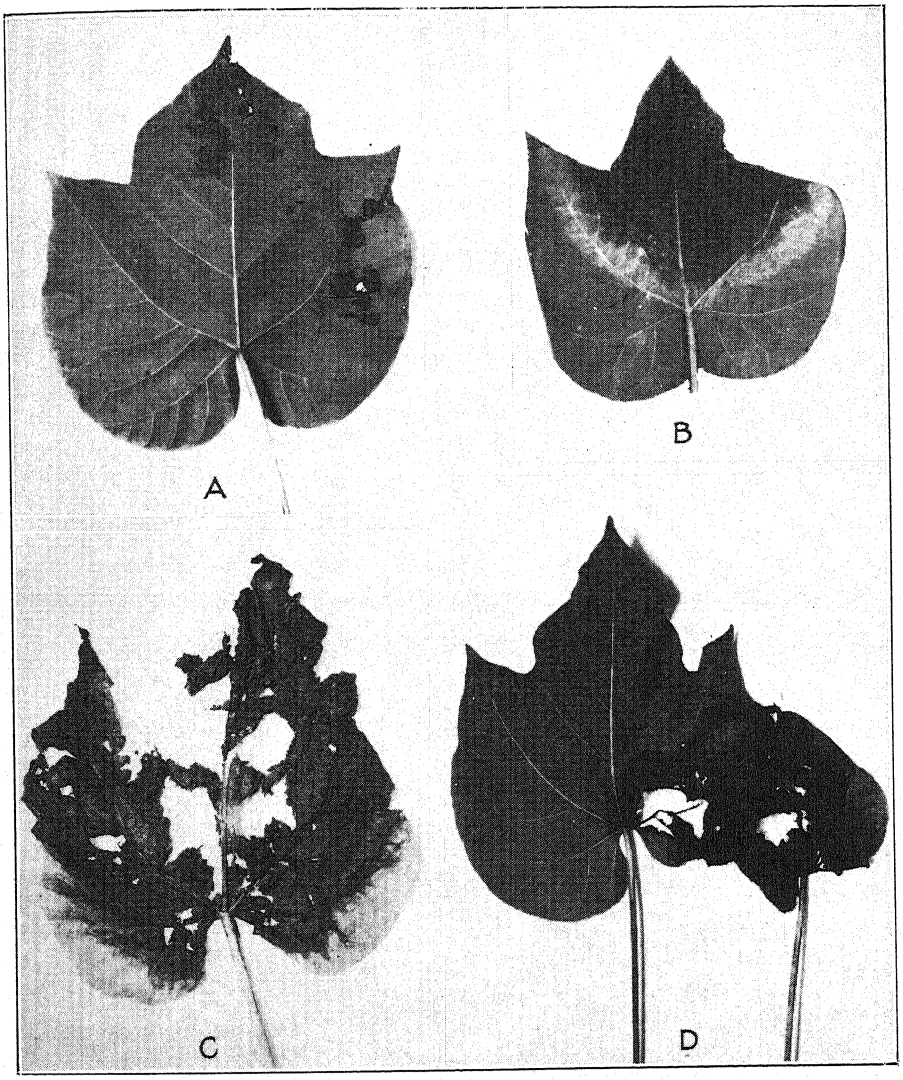


FIG. 1. Progressive stages in macroscopic symptoms of web blight (*Corticium microsclerotia* (Matz) Weber) on tung (*Aleurites fordii* Hemsl.). A. Early stages in the development of primary lesions on tung leaves. B. Extensive superficial mycelial growth spreading from dead to healthy tissues of the leaf. C. Old diseased tung leaf showing the very ragged condition and the very small sclerotia on the petiole and part of the blade. D. Spread of infection by the growth of hyphae from a diseased leaf to a healthy leaf.

phytotic might develop. Weber[2] reported this organism on 24 host plants in Florida, but did not report this disease on tung seedlings.

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PEACH CALICO[1]

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(Accepted for publication October 25, 1943)

Observations in Idaho peach orchards have indicated that variegation of peach leaves, while uncommon, was present in 5 trees. One type is probably of the nature of a chimera, and another is transmissible by bud inoculation. These 2 types of diseases (1, 3) and others (6, 7, 8) have been called calico. The present paper records the writer's experience with peach-leaf variegation in Idaho and describes the virus disease, peach calico.

HISTORICAL

In June, 1939, J. B. Murphy, a grower near Caldwell, Idaho, called the writer's attention to an interesting J. H. Hale peach tree, one branch of which had leaves showing a brilliant yellow-green variegation. Symptoms suggested the name calico (1). Transmission tests using bud wood, designated as P–12, taken from this branch and placed on healthy peach nursery trees at Moscow in the fall of 1939, and in several subsequent trials, indicated that the trouble was bud-perpetuated but not transmissible (Table 1). After these tests and after having observed the original orchard tree again in 1940 (2) and several times later, the condition was regarded as a chimera.

The writer and E. L. Reeves, while examining an orchard near Wilder in August, 1941, found a single small shoot with striking leaf variegation, arising from a main branch near the crotch of a mature Rochester peach tree. Bud wood from this shoot was likewise taken and placed on healthy peach nursery trees at Moscow. This case at first appeared to be similar to the calico, P–12, noted first in 1939, and, although it was reported by this name (3), the collection was listed as P–31.

Two seedling peach trees, budded with P–31 in the fall of 1941, showed in July, 1942, that they had become infected, although the inoculation buds did not grow, and exhibited symptoms similar to those on the original orchard tree (Table 1). Thus, apparently, a virus disease was involved. The original shoot on the orchard tree again showed calico symptoms in 1942, and a small portion of one of the main branches was also affected. Symptoms on fruit were observed first in June, 1943, and are described later.

A peach tree, a few hundred feet from P–31, inoculated in 1940 with western X disease buds, showed in September, 1942, only one spur with calico (P–31) symptoms. It is of interest to note that western X has not been transmitted nor even perpetuated on this tree, although 2 "diseased

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 226.

[2] The writer wishes to express his sincere thanks to L. C. Cochran and E. L. Reeves for their interest in the interpretation of experimental results, field observations, and critical reading of the manuscript. The writer did not have an opportunity to read the pertinent paper of Woods and Du Buy, Phytopath. 33: 637–655, 1943.

buds'' had each borne 2 peaches in 1941 and several in 1942 and 1943. The tree was bud-inoculated with western X again in August, 1942. Sufficient bud wood of this new case was not available for transmission tests, and, when examined in June, and again in September, 1943, no variegation was found on the tree.

In an orchard at Emmett observed in June, 1943, a single leaf on a young tree was found showing symptoms of the nontransmissible or P–12 type of variegation. This particular orchard has a high percentage of trees infected with western X.

In another orchard at Emmett, where 4 trees had been bud-inoculated in 1940 with peach wart, one of the Elberta trees (No. 6, Table 1, p. 25 [5]), showing wart and a slight mottling of the foliage also, bore a leaf with symptoms similar to those of P–31, the transmissible calico.

NAME OF THE DISEASE

Since the name calico had been used by the writer for the nontransmissible leaf variegation or chimera, P–12, and it had also been applied to the virus disease P–31, before the etiology of the latter was known, there is a question as to the proper name to apply. This question was further complicated by the publication by Thomas and Rawlins (8) of the name almond ''calico'' transmissible to peach and cherry, although they had described ''calico'' as the only disease under ''almond mosaics.'' Reeves (7) mentioned a disorder of peach trees observed in Washington and indicated that leaf symptoms appeared similar to those of ''Calico'' referred to by the writer (3). Reeves (7) did not report transmission, but a subsequent communication from him in August, 1943, states that ''positive transmission has been obtained in several instances with the peach disease found in Washington that exhibits leaf symptoms similar to the disease observed on a Rochester peach tree near Wilder, Idaho, listed as P–31 in the Idaho collection.'' The writer has been informed that other cases of peach-leaf variegation occur in the West, but the etiology in all cases is not known. H. E. Thomas, in personal correspondence, states that he does not believe the Idaho P–31 is the same as his calico on almond, but is more like the symptoms he has noted on a transmissible disease of nectarine.

The writer suggests the name peach calico as appropriate for the virus disease illustrated in figure 1, B. Further tests are needed before the relation to other leaf variegation is known.

SYMPTOMS

Symptoms of the calico disease may appear as the leaves unfold, showing in the early stages a mottling or mosaic-like pattern. Later the light-green areas enlarge principally along the veins and the color changes finally to a brilliant-yellow, almost papery-white (Fig. 1, B). Even the twigs become a creamy-white in streaks or non-uniform patterns, and the margins of these streaks or areas may be pink. All leaves may not show symptoms. The

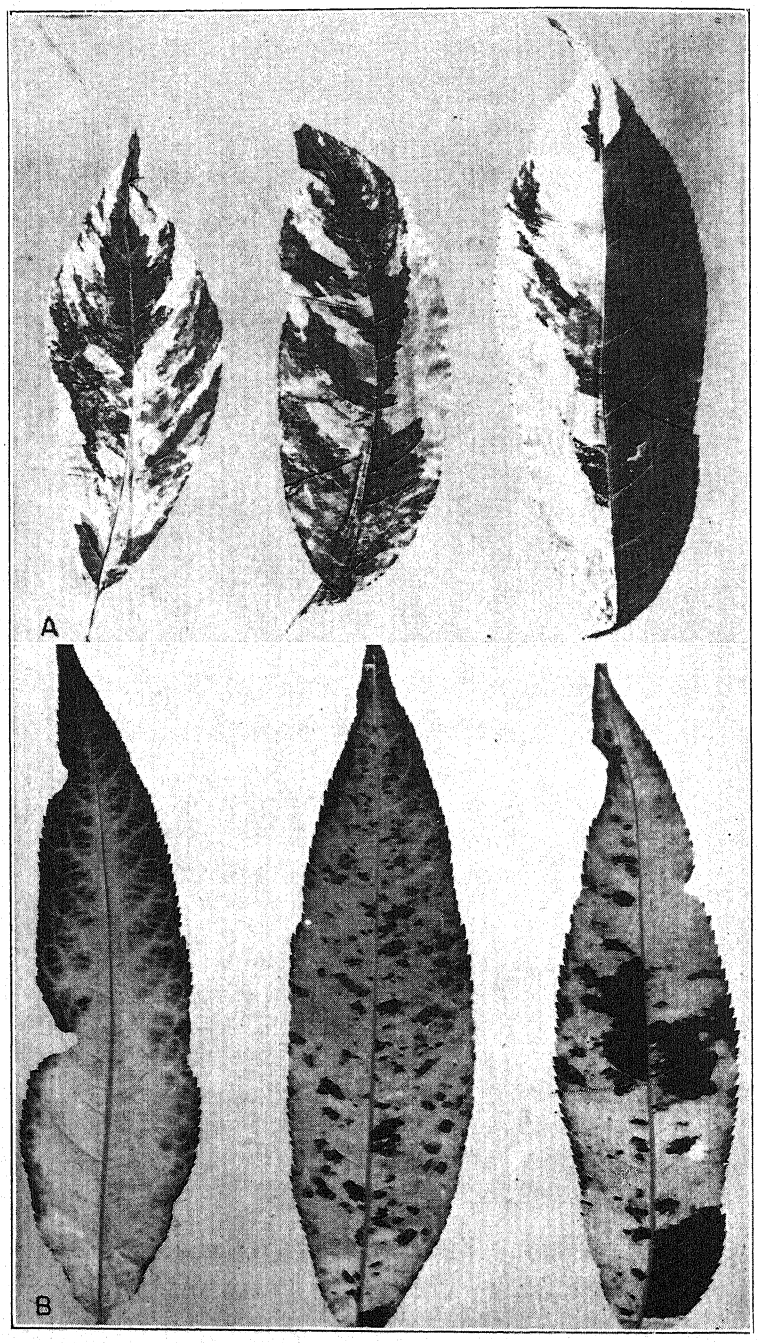


FIG. 1. Peach leaf variegation. A. J. H. Hale leaves affected by a nontransmissible disorder regarded as a chimera (collection P–12). B. Calico, a virus disease on seedling peach in which diseased Rochester buds had been inserted 11 months before (collection P–31).

comparison of calico (P–31) and the chimera (P–12) are shown in figure 1, A and 1, B. Note that in the case of P–12 there are rather sharply delimited areas with at least 3 distinct shades of green, while in P–31 or peach calico the veins are yellow or white and there is a gradual grading from green to yellow to white.

In June, 1943, some of the peach fruits, then about the size of walnuts, showed symptoms. The affected fruits were smaller, shorter and more round than those not affected. The most striking evidence, however, consisted of irregular patches of creamy-white showing through the pubescent surface. In a few cases the affected area was orange shading into dull-red.

TRANSMISSION STUDIES

In table 1 are shown the results of bud-inoculation tests involving cion wood of the chimera P–12 and the calico material P–31. There is no evidence that transmission occurred in the case of tests 1–8 with P–12, and some of the trees are still under observation.

On trees budded with calico P–31 in the fall, symptoms appear early in the spring and are severe on the shoot growth from the inoculation bud, but infection of the stock usually does not become evident until past midsummer. This fact, and the apparent slow spread in the original tree, indicates that the virus does not move rapidly in a tree, and, in case of natural spread in an orchard, considerable time might elapse before the condition would be regarded as serious. Since the virus is readily perpetuated by budding, the use of disease-free bud wood would prevent spread through nursery stock.

It appears from the transmission studies conducted that peach calico is not always transmitted by bud inoculation, at least during the first season of growth after inoculation in the fall.

DISCUSSION

There has been considerable speculation regarding the origin of a virus. Considering the age of the calico-infected orchard tree and other factors, there seems to be only a slight possibility that this certain tree was infected with calico when it was planted in the orchard. There is of course only circumstantial evidence to indicate that this represents a case of a virus originating in peach, but there seems to be some likelihood that such a possibility exists. As more, apparently new, virus diseases are found (or recognized) and described, there seems, as mentioned in the case of peach wart (5), to be additional evidence of new viruses so originating. The information concerning the nature of viruses themselves is such, it seems, as to allow this question to be discussed, although proof may be far in the future. Considering the versatile character some viruses possess, it seems possible to the writer that they may be originating within rather than being contracted by certain host plants. This of course does not mean that life may "originate" by itself. It may mean, however, that the conditions necessary for the formation of virus entities occasionally occur in nature and need not necessarily

TABLE 1.—*Results of transmission tests by budding with cion wood from peach trees affected with leaf variegation (a) chimera (b) calico. Moscow, Idaho. 1939–1943*

Test No.	Source of buds	Stock variety	No. of buds inserted	Date budded	Condition of inserted buds	Results on stock
				June 1940		
a–Chimera 1	P–12 Caldwell	Slappey	6	10/4/39	All buds dead	No transmission
2	"	"	"	"	" " "	Tree removed 1942. No transmission
3	"	"	"	"	" " "	" " " " "
				August 1941		
4	P–12 Caldwell	Peach seedling	3	9/26/40	All buds dead	No symptoms. " "
5	"	" "	"	"	" " "	" " " "
6	"	" "	"	"	One bud dead. Two vigorous shoots	One cion shoot, no symptoms. No transmission. One cion shoot, typical symptoms. No transmission
7	"	" "	2	"	Both buds dead	No symptoms. No transmission
8	"	" "	"	"	" " "	" " " "
				August 1942		
b–Calico 1	P–31 Wilder	J. H. Hale	3	9/15/41	All buds dead	No symptoms, 1942. No transmission. Tree dead, 1943
2	"	" " "	"	"	" " "	No symptoms, 1942. No transmission. Tree dead, 1943
3	"	" " "	"	"	" " "	No symptoms, only seedling shoot. No transmission
4	"	Peach seedling	"	"	" " "	Typical symptoms. Transmission
5	"	" "	"	"	" " "	" " "
6	"	J. H. Hale	"	8/24/42	One bud dead. One bud alive. One vigorous shoot	Severe symptoms on shoot from inoculation bud. No transmission. 10/7/43
7	"	" " "	"	"	Two buds alive. One shoot	Severe symptoms on shoot from inoculation bud. No transmission. 10/7/43
8	"	" " "	"	"	One bud dead. Two shoots	Severe symptoms on shoot from inoculation bud. No transmission. 10/7/43

TABLE 1.—(*Continued*)

Test No.	Source of buds	Stock variety	No. of buds inserted	Date budded	Condition of inserted buds	Results on stock
b–Calico (Cont.)						
9	"	Elberta	4	"	Two buds dead. One bud alive. One shoot	Severe symptoms on shoot from inoculation bud. No transmission. 10/6/43
10	"	"	"	"	Three buds dead. One bud alive	One affected leaf on stock. Positive transmission. 10/6/43
11	Tests No. 4, 5, calico, Moscow plots	Italian prune	3	8/29/42	Three buds alive	No definite evidence of transmission. Leaf color abnormal. 10/7/43
12	"	"	"	"	Three shoots	Symptoms on shoots, but not certain of transmission. 10/7/43
13	"	Moorpark	"	"	One bud dead. Two buds alive	No evidence of transmission. 10/6/43
14	"	"	"	"	Two buds alive. One shoot	Symptoms severe on shoots from inoculation bud. No evidence of transmission. 10/6/43
15	"	May Duke	"	"	All buds alive early but died later	No transmission noted. 10/6/43
16	"	Bing	"	"	All buds dead	" " " "
17	Tree, test No. 4	Ne Plus Ultra almond	Inarched, 2 contacts	April, 1943	Both contacts positive	No evidence of transmission. 10/7/43

be coupled with a progenitor as such. It is readily agreed that "sports" or mutants arise, and the writer considers it possible that the difference between perpetuated abnormalities, such as P–12 or chimera and transmissible diseases as P–31 or calico, may be, after all, fundamentally rather slight. If the agent responsible for the failure of chlorophyll to develop in nontransmissible variegations like P–12 could move out of affected cells, it would become a transmissible entity—one into which P–31 would fit. The development of peach calico symptoms in some of the older leaves gives evidence that some agent is responsible for the destruction of chlorophyll already formed. Some workers have suggested that viruses might be disorganized chromatin material, which, once outside its normal cell function, is able to reproduce itself at the expense of the cell. The presence of only one affected leaf on the stock of a bud-inoculated tree (No. 10, Table 1) suggests an erratic, unpredictable behavior for the virus causing peach calico.

The period of incubation of some fruit virus diseases is so long that at this stage of our knowledge the supposedly new viruses may, in reality, be delayed appearances. The rather sudden increase in the number of "odd" virus diseases still leaves a possibility of "spontaneous" origin of plant viruses.

It might be true, too, that in plant diseases we have neglected the possibility of wind- or air-borne viruses, such as are reported to occur in animal pathology. These new cases might be the establishment of an unsuspected virus such as one from an herbaceous plant in a woody plant. This hypothetical assumption, however, might not even, if proved, settle the point as to whether the virus is "new."

The writer does not wish to infer that the leaf variegations noted, necessarily have any connection with the virus diseases, peach wart and Western X. The observation might, however, have more significance than the mere recording.

The above discussion, of course, has not mentioned all the important points in a problem of this nature, but it may serve to indicate that there still are many questions to answer as to where "new" viruses come from. The occurrence of the disease, peach calico, has been of great interest to the writer from this standpoint.

SUMMARY

Variegation of peach leaves on 5 trees in Idaho has been shown by bud inoculation to be of at least 2 types. One, nontransmissible, is regarded as a chimera and is characterized by irregular yellowing in which 3 rather distinct shades of green are exhibited. The other type, called calico, characterized by extensive yellowing, finally produces a papery-white leaf and twig tissue. It is transmissible. Affected fruit is shorter, more round and shows creamy-white to red patches. Historical facts and transmission tests are reported and the two types of disease are compared. The question regarding the possible origin of the virus is discussed.

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DISSEMINATION OF A PEACH MOSAIC

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The disease initially referred to[1] as the Winters peach mosaic and here designated as yellow bud mosaic (*Inops consilii*) has been found in 11 additional orchards in the Winters District of Solano and Yolo Counties, California. The change to the more descriptive name is based on an early season sequence of symptoms in which many buds on the severely affected branches push out to a few millimeters in length at the beginning, then remain pale yellow and virtually at a standstill on otherwise bare branches for as much as several weeks. These buds later may die or slowly develop a tuft of small somewhat distorted leaves (Fig. 1, C). In tree-by-tree examination of 10 of these orchards, infected trees were found to be not scattered more or less at random as was expected but in compact groups (Fig. 1, A and B) indicating that the transfer of virus usually is from one tree to the next adjacent rather than over greater distances. Occasional foci of infection are found at considerable distances from any apparent source, but the spread from these foci is in turn almost invariably to immediately adjacent trees. This distribution in the orchards is quite unlike any known to the writers for other fruit-tree virus diseases, including the similar mosaic of peach in Colorado.[2] With the buckskin disease, for example, in orchards of the Winters and several other districts of the State infected trees usually are widely separated and often without any apparent relation to source of inoculum (Fig. 1, B).[3]

The three varieties, Elberta, Lovell and Muir, commonly grown in the Winters District, are all distinctly susceptible to yellow bud mosaic and seem to become infected with about equal facility under orchard conditions.

Among the stone fruits other than almond, apricot, and peach, only plums (*P. domestica*) have been seen under extreme exposure to natural inoculation. No mosaic symptoms have been seen in the plums that seem to be related to the disease in peach nor have any been produced by artificial inoculation of *domestica* plums. None of the 7 native species of *Prunus* recorded for the State has been found in the vicinity of affected orchards.

Since apricots and almonds are the leading fruits of the district and both are susceptible to the yellow bud mosaic, the relation of these to the disease in peach is of interest.

The virus seems to spread readily from peach to apricot and from apricot to peach (Fig. 1, B) but the apricot is so little injured that symptoms are often entirely lacking, especially in older trees. The orchard shown in part

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[2] Bodine, E. W. Peach mosaic disease in Colorado. Colorado Agr. Exp. Stat. Bull. 421: 1–11. 1936.

[3] Thomas, H. Earl, and C. Emlen Scott. Prevalence of buckskin in peaches. U. S. Dept. Agr. Pl. Dis. Rptr. 27: 292–293. 1943.

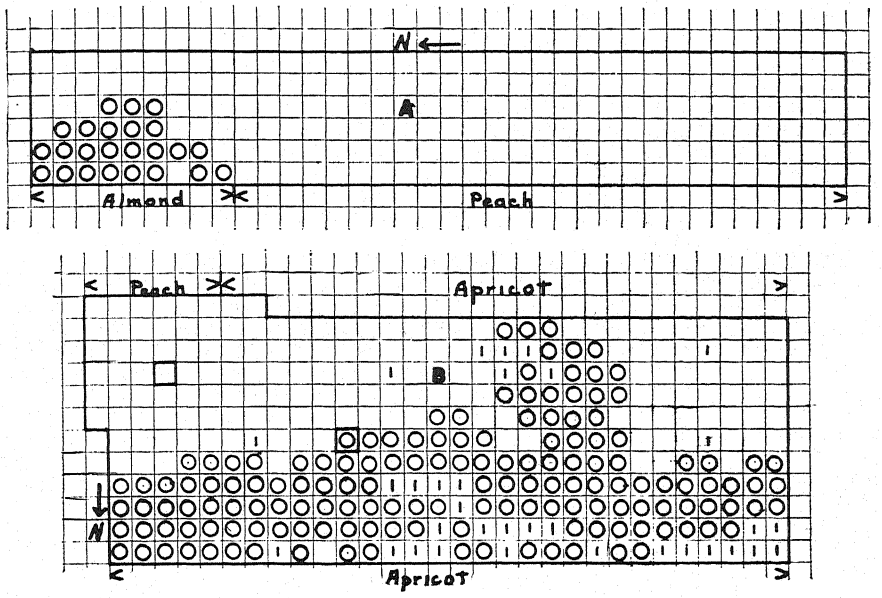




FIG. 1. A. Spread of yellow bud mosaic from almond to peach. B. Spread from apricot to peach. Circles indicate infected peach trees, dashes represent missing or replanted trees. The two squares in B bordered by heavier lines represent trees infected by buckskin. C. Severely infected Lovell peach trees photographed in June.

in figure 1, B, is one of several in which a clear relation between apricots and infection of peaches is indicated. This block of 14-year-old Lovell trees is bordered on the north by a block of apricots beyond which a block of Muir trees had an increasing number of infected trees from 1936 and probably earlier until it was removed in 1942. Most of the south border of this Lovell block is also apricots, but the map indicates that these are not supplying inoculum. The one group of infected trees, which reaches the south border, probably represents a single instance of dissemination over a greater distance than usual. This surmise is supported by the presence of several replants, now about 5 years old, near the center of this group. The disease was noticed in this block for the first time in 1940, but probably was present for some time prior to that year. The map shown in figure 1, B, was made in June, 1943.

Several foci of infection in other peach orchards were found in which the only infected trees were in outside rows adjacent to apricots.

The almond, on the other hand, may be considerably injured by the disease (intermediate between apricot and peach) but is much more likely to escape infection. For example, an almond block of about 15 acres now in the 5th growing season was interplanted among heavily infected peach trees a year before the peaches were removed. On one border is an apricot block that has had a considerable number of infected trees throughout the 5-year period. To date no evidence of infection in this block of almonds has been found.

At least one case has been seen, however (Fig. 1, A), in which almond seems to be clearly a source of the virus for peach. Six rows of 36 trees each (216 trees) about 10 years old lie adjacent to a short block of old almond trees several of which in the 2 or 3 rows nearest these peach trees bear the type of symptoms characteristic of the disease in almond. The remainder of that side of the peach block is bordered by older peach trees. It seems obvious from figure 1, A, that the virus has spread from the almonds to the peaches and this evidence is supported by the fact that the most severely affected peaches are nearest to the almonds. Two other sides of this almond block are bordered by older peach trees, but no infected peaches or almonds were found at these boundaries.

In contrast with the orchards discussed above are others with no apparent external source of inoculum. In the latter, there are typically fewer infected trees in 1 or 2 outside rows than in those farther from the borders. This seems true whether the orchard be adjoined by a highway or by another orchard.

Several orchards were found in which the disease undoubtedly is perpetuated by replanting peach trees among or adjacent to older trees. In one such case, infected trees were seen ranging in age from 2 to 30 or more years.

Since the vectors of nearly all virus diseases of fruit trees remain not only unknown but without even a good clue, it may not be altogether futile

to speculate as to the possible significance of the field observations as bearing on the type and habits of the vector. The observed facts seem to indicate that the vector has a very limited range of movement, is readily attracted to peach and apricot, distinctly less so to almond, and has a tendency to shun the border rows of peach blocks. Less certain is a seeming preference for low areas in the orchards.

One of the writers (Freitag), in 1938, tested about 20 species of insects as possible vectors of the yellow bud virus on 332 inoculated trees. All results were negative.

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ASSOCIATION OF XANTHOMONAS PHASEOLI AND THE COMMON BEAN-MOSAIC VIRUS, MARMOR PHASEOLI. I. EFFECT ON PATHOGENICITY OF THE SEED-BORNE INFECTIVE AGENTS

FLORENCE HEDGES[1]

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INTRODUCTION

The discovery of masked virulent *Xanthomonas phaseoli* (E. F. Sm.) Dowson in the inoculum used in testing hybrids of common bean (*Phaseolus vulgaris* L.) for mosaic resistance, led the writer to undertake the studies here reported. A preliminary report (14) has been published. Investigations of this kind are important for the following reasons:

1. The breeding of resistant varieties is one of the most effective means of combating plant diseases, and is being widely applied to beans, with excellent results. The procedure involves the testing of the parents and progeny for resistance by inoculation with the infective agent in question.

2. The type of pure-culture inoculation employed with bacteria and fungi has not been possible with the viruses, as investigators have yet to discover a means of cultivating them on a nonliving substratum (25). The common practice among plant pathologists working with virus diseases has been, therefore, to use the juice of infected plants as inoculum.

3. An unsuspected association of two infective agents may occur in the test plant if, as in the case under consideration, a second parasite is masked in the plant tissues the juice of which is used as inoculum. Both the virus of the common bean mosaic, *Marmor phaseoli* Holmes, and *Xanthomonas phaseoli* are seed-borne, and are often associated in nature.

4. The association of infective agents may result in complications that would go far to invalidate the interpretation of results by the investigator who had introduced one of them unwittingly along with his inoculum.

Pasteur's early work on fermentations (20) and Pasteur and Joubert's (21) discovery that *Bacillus anthracis* could be inhibited both *in vitro* and *in vivo* by the simultaneous inoculation with various common bacteria were the forerunners of studies of microbial associations in all fields of microbiology. The literature on this subject has now assumed voluminous proportions and has been reviewed recently by Waksman (33, 34).

It has been experimentally shown that association, though sometimes without apparent effect, often results in mutual or unilateral antagonism or stimulation. The practical importance of this is obvious and has been voiced by Waksman in the second of his reviews (34), in which he says: "It is possible we are finally approaching a new field of domestication of micro-

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organisms for combating microbial enemies of man and his domestic plants and animals."

One of the best-known therapeutic measures based upon the principle of the antagonism of micro-organisms is the malaria treatment of syphilis. Following the brilliant work of Wagner v. Jauregg (15) for which he received the Nobel prize in 1927 (3), this method has become a routine in mental hospitals (12).

In more recent years the soil as a source of organisms antagonistic to pathogens of man, animals, and plants has attracted considerable attention. Foremost in the field are Waksman and Woodruff (35, 36, 37), who isolated a soil *Actinomyces* (*Act. antibioticus*) active against a great variety of bacteria and fungi, pathogenic and nonpathogenic, and Dubos (8, 9, 10, 11), who obtained from the soil a bacillus secreting a bactericidal agent, gramicidin. Gramicidin is active *in vitro* and *in vivo* against Gram-positive bacteria, including pneumococci and streptococci. An extract of the bacillus in question protects white mice against infection with large numbers of virulent pneumococci. Little, Dubos, and Hotchkiss (18), working with an oil mixture of gramicidin, effected complete cures in 84 per cent of cases of bovine mastitis, some of which were of a severe chronic nature.

Penicillin, the chemical agent of such promise as a bacteriostatic agent, was isolated by Fleming (13) following his observation of the lysis of staphylococcus colonies surrounding a Penicillium contaminant in one of his plates.

Examples of antagonism are not lacking in the field of plant pathology. Brown and Quirk (6), in their work on the influence of bacteriophage on *Agrobacterium tumefaciens* (Erw. Sm. and Towns.) Conn, found that the largest and greatest number of plaques due to the lytic principle could be obtained on beef agar plates and slants with filtrates of carrot rotted as a result of pure-culture inoculation with *Bacillus carotovorus* Jones. The presence or absence of plaques was linked with oxidation and pH potentials. This idea of chemical-physical stimulus was thus expressed by Erwin F. Smith (32) in a paper on the production of tumors in the absence of parasites: "All tumors, so far as they are due to parasites, must be assumed to be due to the chemical-physical action of the by-products of the metabolism of these parasites just as most communicable diseases are due not to the parasites themselves but to their toxins."

Two antagonistic bacteria occurring *in vivo* have been reported by Adam and Pugsley (1). They frequently found associated with *Pseudomonas medicaginis* var. *phaseoli* (Burkh.) Stapp and Kotte in the host a nonpathogenic yellow organism that, when mixed with the pathogen at the time of inoculation, retarded the rate of development of the very destructive bean halo blight.

Ark (2) has isolated two soil bacteria that have proved strongly antagonistic to 16 phytopathogenic bacteria and 6 fungus plant pathogens.

In the plant-virus field cross immunity has been demonstrated with strains of a given virus in numerous groups; *e.g.*, Kunkel (16) has shown

that protection is afforded *Nicotiana sylvestris* Spegaz. and Comes against the necrotic type of aucuba mosaic virus by earlier inoculation with ordinary tobacco mosaic virus or with either of two attenuated strains of aucuba mosaic virus. Equally striking is his work (17) showing that the viruses of peach yellows and little peach produce reciprocal immunity in the host.

Another example in this field is the acquired immunity of *Zinnia elegans* Jacq. from cucumber mosaic. Price (23) found that a necrotic type strain could not infect mottled leaves of plants infected with any one of 4 strains of cucumber mosaic. An excellent review of the subject of acquired immunity from plant-virus diseases is given by Price (24).

An outstanding example of the stimulating effect of one infective agent upon another is swine influenza. Shope (26) in 1931 cleared up the mystery of this highly contagious disease which made its appearance at the time of the great pandemic of human influenza in 1918 and appears to be closely related to it (27, 28). He proved that it was caused by a bacterium and a virus acting synergistically, the first such case on record among the diseases of animals or man. The bacterium, *Hemophilus influenzae* var. *suis* Shope, though constantly associated with the disease, proved to be nonpathogenic when administered alone; the virus filtrate alone, on the other hand, produced an extremely mild though contagious ailment. A mixture of the virus filtrate and pure cultures of the bacterium caused a prostrating illness, an extreme pneumonia identical clinically and pathologically with swine influenza.

An example of synergism from the plant kingdom is a bacterial disease of ivy in which Burkholder and Guterman (7) found a nonpathogenic bacterium to be associated with the pathogen *Xanthomonas hederae* (Arnaud) Dowson, and to produce an accelerating effect upon it. Plates were poured from lesions artificially produced by a mixture of the two organisms. Plates from small spots contained an approximately equal number of the two types; those from large lesions, on the other hand, contained a much greater number of the accelerating bacterium than of the pathogen.

Brierley (4, 5) has presented evidence of an example of synergism in the plant virus field. He showed associated in the flecked mosaic of Easter lilies, a virus of the cucumber mosaic group and a virus causing breaking in tulips.

The foregoing illustrations chosen from the field of infectious diseases, animal and plant, relative to interactions of associated organisms suffice to make clear their significance. They justify Waksman's statement (34) previously quoted and show why, in testing breeding stocks and hybrids for disease resistance, it is imperative to know whether more than one organism is present in the inoculum.

HISTORY OF THIS ASSOCIATION

The circumstances leading to the discovery of the association under discussion were as follows: In the course of mosaic-resistance tests being carried

on by W. J. Zaumeyer at The Plant Industry Station, Beltsville, Maryland, the attention of the writer was called to typical *Xanthomonas phaseoli* lesions on a very high percentage of plants of a bean hybrid, later known as U.S. No. 5 Refugee. These had been inoculated about 3 weeks earlier with bean virus 1 from seed-infected bean plants. The primary leaves of the hybrid in question had been rubbed with the juice of trifoliate leaves of Stringless Green Refugee showing typical common bean mosaic symptoms but no bacterial lesions.

The inoculated hybrid, which was mosaic-resistant, showed no mosaic symptoms, but, instead, widespread bacterial infection on the rubbed primary leaves. This phenomenon had been observed in a number of earlier similar inoculation experiments. Furthermore, a great number of typical *Xanthomonas phaseoli* lesions had been observed also on the inoculated primary leaves of mosaic-resistant Corbett Refugee and Robust beans tested by Zaumeyer with 3 clover mosaics: 1. Bad bacterial infection occurred on Corbett Refugee inoculated with alsike clover mosaic. 2. There was slowly developing bacterial infection on Corbett Refugee inoculated with alsike clover mosaic IV. 3. Mild bacterial infection on Robust followed inoculation with red-clover mosaic. All of these clover mosaic viruses had been passed through bean previous to these inoculations. There had been no sign of bacterial infection on these bean plants used as inoculum for the tests here described. From all three sets of clover-mosaic-virus inoculations the writer isolated "typical" *Xanthomonas phaseoli* from the inoculated primary leaves of bean.

Subsequently, on a number of occasions, the writer has had similar experience when using as inoculum trifoliate leaves of Stringless Green Refugee grown from seed infected with bean virus 1.

From the bacterial lesions on the primary leaves of the aforementioned hybrid (U.S. 5 Refugee) there was no difficulty in isolating "typical" *Xanthomonas phaseoli.* This was designated *X. phaseoli* "M-associate" (that is, mosaic-associate), and was the isolate thereafter used for the majority of the experiments the first year of this investigation.

Whence came the bacteria? In an attempt to answer this question, plates were poured from trifoliate leaves with typical mosaic symptoms but without signs of bacterial infection, *i.e.,* leaves resembling those used as inoculum for the aforementioned bean hybrid (U.S. 5 Refugee). From such leaves was isolated a comparatively small number of colonies of a yellow organism which was later tentatively styled *Xanthomonas phaseoli* variant "M-associate." This variant[2] did not resemble the so-called "typical" *X. phaseoli,* either in the isolation plates or on potato cylinders. It was, however, capable of producing a bacteriosis similar to some types of *X. phaseoli* infection and appeared, in most cases, to revert within the plant to the "typical" *X. phaseoli.*

[2] This variant produces bright-yellow colonies with a wide, cross-hatched opalescent margin on pH 7.0 beef-infusion agar plates, and makes relatively scant restricted deep-yellow growth on potato, a marked contrast to the characteristic copious ± fluid growth of "typical" *X. phaseoli.*

At this early stage in the investigation it seemed apparent, therefore, that the bacteria had been masked in the mosaic inoculum and introduced unwittingly therewith into the hybrid being tested for mosaic resistance.

Since these early isolations, "typical" *Xanthomonas phaseoli* has been obtained repeatedly from Stringless Green Refugee trifoliate leaves showing typical mosaic infection and no bacterial lesions. It also has been isolated from Corbett Refugee trifoliate leaves with neither mosaic symptoms nor bacterial lesions. In all these later instances, however, the writer had knowingly introduced *X. phaseoli* or its variant into the plants. This was done by inoculating bean seedlings with a mixture of mosaic-virus-infected juice and pure single-colony cultures of the bacterium.

The masking of *Xanthomonas phaseoli* also occurs in leaves and stems of Stringless Green Refugee free from mosaic infection (unpublished work), and, hence, is not dependent on the presence of the virus. But the fact that this seed-borne bacterial parasite is not infrequently masked in virus-infected tissues raises many questions and introduces an element of uncertainty into the present methods of testing bean breeding stock, varieties, or hybrids for mosaic resistance. It becomes of the utmost practical importance, therefore, to determine whether antagonism or stimulation of one or both of the two infective agents results from their association in the host.

MATERIALS AND METHODS

Hosts

The bean varieties used as hosts in these investigations were Stringless Green Refugee (susceptible to both the virus and the bacterium) and Corbett Refugee (mosaic-immune, *Xanthomonas phaseoli*-susceptible—one of the parents of the hybrid later known as U.S. No. 5 Refugee). Unfortunately, there is no known bean variety resistant to *X. phaseoli.*

Seed Stock

The same line of Stringless Green Refugee was used throughout. The seed originally obtained from a reputable grower was assumed to be a pure line. The genetic purity of the line throughout these studies cannot be guaranteed, although no apparently off-type plants were noted. The seed used in these experiments was collected from healthy plants in the breeding plots grown by W. J. Zaumeyer at Greeley, Colorado, or from the writer's uninoculated check plants grown therefrom. The same was true of Corbett Refugee.

Inocula

The following infective materials were used as inocula in the course of these investigations:

1. Mosaic virus (bean virus 1, *Marmor phaseoli*)—juice from trifoliate leaves of plants grown from infected seed and showing typical symptoms of the common bean mosaic.

2. *Xanthomonas phaseoli* "M-associate"—isolated from mosaic-infected plants at various times.

3. *Xanthomonas phaseoli* variant "M-associate"—an atypical, mildly pathogenic yellow form found (1) masked in typical mosaic-infected trifoliate leaves and (2) in the petiole of a typical mosaic-infected trifoliate leaf in the leaf-blade of which "normal" *X. phaseoli* was masked. It sometimes reverted to "typical" *X. phaseoli* in the host.

4. *Xanthomonas phaseoli*—a number of isolates with no history of association with the virus.

5. Mosaic virus plus the bacterium or its yellow variant—virus-infected plant juice to which single-colony cultures of the bacterium had been added: designated M + P and M + Pvar.

6. Juice from plants artificially infected with both mosaic virus and bacterium in a series of direct passages from plant to plant with no intervention of culture media; designated (M + P) and (M + Pvar).

In addition to the above, the writer has tested for pathogenicity bacterial forms encountered from time to time in the isolation plates from plants inoculated with the combined virus and bacterium.

Isolations of bacteria were made on pH 7.0 beef-infusion agar. When plates were poured from trifoliate leaves showing no bacterial lesions, it was necessary to use a large quantity of the plant material for plating.

Preparation of the Inocula

Virus. In the earlier experiments the virus inocula were prepared by macerating the infected trifoliate leaves in a sterilized mortar and expressing the juice. Later the infected material was merely crushed in the gauze pad used to apply the inoculum. In every case the leaves were collected and crushed just before using.

Bacterium. The bacterial inocula consisted of sterile distilled water suspensions of the growth in young single-colony cultures grown on steamed potato cylinders.

Mixed Inocula. When the bacteria were mixed with virus-infected juice, no preliminary water suspension of the bacterial growth was made (with a single exception), since bean virus 1 will stand but little dilution. The mixture was not allowed to stand, but was used immediately. Sometimes the potato cylinders themselves were macerated and incorporated with the bacterial growth in the mixed inoculum.

The juice of infected plants containing both virus and bacterium was obtained by crushing the trifoliate leaves or plant tops in the inoculating pad. (See Part II Serial passages.)

Inoculation Methods

The inoculations in these investigations were made on young seedlings having, as a rule, the primary leaves only. In the early experiments the leaves were supported by a wooden block and the inoculum was applied to

the under surface with a gauze pad. Sufficient pressure was exerted to cause considerable injury. Later, a pasteboard tag was substituted for the wooden block, which resulted in less severe but sufficient injury. Carborundum was not used in the earliest experiments, but later was tried for a time. It was found to be quite unnecessary, and its use was discontinued.

All inoculations were made in a greenhouse or in a screened house without glass. Plants were shaded for about 24 hours following inoculation.

Uninoculated plants were always held as checks. These were isolated from the inoculated plants. They were not rubbed with distilled water, which has no effect on bean foliage, or with apparently healthy trifoliate leaves, lest the latter contain a masked parasite. In these studies, one of the main purposes of the checks was to determine whether there was seed infection.

Abbreviations

M—Mosaic virus
P—*Xanthomonas phaseoli*
Pvar—a yellow variant of *Xanthomonas phaseoli*
(M + P)—juice from serial passages started with juice containing the virus plus "typical" *X. phaseoli*
(M + Pvar)—juice from serial passages started with juice containing the virus plus the yellow variant of *X. phaseoli*
M-assoc.—bacterial isolate from a mosaic-infected plant

PART I

EARLY ANGLES OF APPROACH

EARLY GREENHOUSE EXPERIMENTS

Inoculations were made in the greenhouse the latter part of April in order to test the pathogenicity of the original bacterial isolates which had been found associated with the common bean mosaic (bean virus 1). The following inocula were used:

1. Mosaic virus (bean virus 1, *Marmor phaseoli*); expressed juice from trifoliate leaves of seed-infected plants.
2. *Xanthomonas phaseoli* "*M-associate.*" Isolated 6 weeks before from typical *X. phaseoli* lesions on primary leaves of the hybrid later known as U.S. No. 5 Refugee, which had been inoculated with bean virus 1, but showed no virus symptoms; young transfers on steamed potato cylinders from stock cultures on the same medium, kept in the refrigerator after the initial growth period of about 2 weeks. For Stringless Bean Refugee the whole culture, including the potato cylinder, was used; for Corbett Refugee the bacterial slime was scraped off and used undiluted.[3]
3. *Xanthomonas phaseoli variant* "*M-associate.*" Masked in Stringless Green Refugee trifoliate leaves showing typical mosaic symptoms; isolated 11 days previously; young transfers on steamed potato cylinders as above.
4. *Mosaic virus + Xanthomonas phaseoli* "*M-associate.*" (Inoculum 1 + inoculum 2.)
5. *Mosaic virus + Xanthomonas phaseoli variant* "*M-associate.*" (Inoculum 1 + inoculum 3.)

Ninety-six plants that had not yet developed their trifoliate leaves were inoculated, 16 of each of the 2 varieties with the virus, and 8 with each of the other 4 inocula listed above.[4] Fifty uninoculated plants were held as checks.

[3] Later both varieties were tested with and without the incorporation of the potato cylinder in the inoculum. The results obtained from the two methods were the same.

[4] It would have been desirable to have had more plants in these early experiments, but the available amount of seed stock with a known history was limited at this time.

Virus Infection

Most unexpected and thought-provoking results were obtained from these early experiments. Stringless Green Refugee plants, inoculated with the bacterium, *Xanthomonas phaseoli* "M-associate" (inoculum 2), showed typical mosaic symptoms on newly developing trifoliate leaves in 6 days. In 25 days, 75 per cent of the plants were similarly infected. This was as high a percentage of virus infection as occurred in the plants inoculated with the virus-bearing plant juice (inoculum 1) alone.

Similar results were obtained on plants inoculated with *Xanthomonas phaseoli* variant "M-associate" (inoculum 3). The first mosaic symptoms appeared slightly earlier, that is in 4 days, and there was 75 per cent infection in 12 days. (Table 1.)

Both *Xanthomonas phaseoli* "M-associate" and the yellow variant appeared to carry the mosaic virus and produce typical mosaic. The virus apparently persisted in steamed potato cultures of *X. phaseoli* for 6 weeks, although in the expressed juice of mosaic-infected plants it loses its virulence in 1 to 2 days at room temperature.

It was carried apparently likewise in a virulent form by the variant for 11 days.

In this connection, it is interesting to note that Silber (29, 30, 31) and his colleagues preserved the vaccine virus for over 1½ years in yeast cultures at room temperatures. The virus also was adsorbed by and grew on Staphylococcus. Poppe and Busch (22), using their methods, maintained the virus of foot and mouth disease through 60 passages on *Torula rubra.*

The two sets of inocula (inocula 4 and 5), each consisting of a mixture of virus-bearing plant juice with one of the two bacterial isolates, had a slightly shorter incubation period. They produced on Stringless Green Refugee some mosaic in 3 days, as did also the virus-bearing plant juice (inoculum 1) alone—an extremely short incubation period for bean virus 1. In 25 days the virus-bearing plant juice + variant "M-associate" (inoculum 5) had produced 100 per cent mosaic infection, the virus-bearing plant juice alone had produced 75 per cent mosaic infection (Table 1, inoculum 1).

The recorded increase in mosaic after 12 days in all except set 3 (Table 1) is accounted for by the fact that only plants with *unmistakable* signs of typical common bean mosaic were entered in the table, and the majority of the additional infected plants recorded the 25th day had been labeled "possibly infected" on the 12th day, *e.g.,* the 4 additional plants in the virus set.

The writer does not believe that there was any infection due to aphids. Neither the insects themselves nor traces of their work were observed at any time.

No mosaic symptoms appeared on the resistant Corbett Refugee in any of the sets (Table 2). The checks remained free from virus infection.

Bacterial Infection

In regard to bacterial infection, the results on the two bean varieties, both *Xanthomonas phaseoli*-susceptible, were as follows:

Inoculum	Visible bacterial infection produced					"Typical" *X. phaseoli* isolated from mosaic-virus-infected trifoliate leaves
	4 days	6 days	12 days	20 days	25 days	
			Per cent	*Per cent*	*Per cent*	
1. Virus-bearing plant juice	0	0	0	0	0	0[c] (plated June 6 & 14)
2. *X. phaseoli* "M-associate"	+	+	100 (excellent infection on inoculated primary leaves)	100 (inoculated leaves dead)	100 (stem infection in 1 plant)	0[c] (plated June 6)
3. *X. phaseoli* variant "M-associate"	0	0	0	0	0	0[c] (plated June 6)
4. Virus-bearing plant juice plus *X. phaseoli* "M-associate"	0	+	100 (scattering lesions on inoculated primary leaves)	100 (all inoculated leaves dead or dying)	100 (no stem infection observed)	*X. phaseoli* masked in trifoliate leaves showing typical mosaic; isolated June 6[c]
5. Virus-bearing plant juice plus *X. phaseoli* variant "M-associate"	0	0	0	0	0	0[c] (plated June 6)

TABLE 1.—*Inoculations April 26, on Stringless Green Refugee bean plants susceptible to mosaic and to Xanthomonas phaseoli*

Inoculum	Mosaic produced:[a] number of plants[b] and percentage						
	3 days	4 days	6 days	8 days	12 days	20 days	25 days[a]
			No.—%	*No.*—%	*No.*—%	*No.*—%	*No.*—%
1. Virus-bearing plant juice	+	+	7/16[b]—44¾	8/16—50	8/16—50	9/16—56+	12/16—75
2. *X. phaseoli* "M-associate"	0	0	1/8—12½	2/8—25	2/8—25	3/8—37½	6/8—75
3. *X. phaseoli* variant "M-associate"	0	+	3/8—37½	4/8—50	6/8—75	6/8—75	6/8—75
4. Virus-bearing plant juice plus *X. phaseoli* "M-associate"	+	+	3/8—37½	3/8—37½	3/8—37½	4/8—50	6/8—75
5. Virus-bearing plant juice plus *X. phaseoli* variant "M-associate"	+	+	3/8—37½	3/8—37½	6/8—75	6/8—75	8/8—100

[a] The numbers recorded indicate plants showing *unmistakable* signs of typical common bean mosaic. The majority of the additional infected plants recorded the 25th day were marked "possibly infected" on the 12th day, *e.g.*, all 4 of the additional infected plants in the virus set.

[b] Numerator represents number of plants infected. Denominator indicates total number of plants inoculated.

[c] Compare table 3 showing isolation of S opaque white colonies.

TABLE 2.—*Inoculations April 29, on Corbett Refugee bean plants (mosaic-immune variety)*

Inoculum	Mosaic produced			Visible bacterial infection produced					"Typical" *Xanthomonas phaseoli* isolated
	12 days	22 days	32 days	4 days	9 days	18 days	22 days	32 days	
						Per cent	*Per cent*	*Per cent*	
1. Virus-bearing plant juice	0	0	0	0	0	0	0	0	No plates poured
2. *Xanthomonas phaseoli* "M-associate"	0	0	0	+ (considerable infection on inoculated primary leaves)	+ (good infection on inoculated primary leaves)	100 (all inoculated leaves dead or dying)	100 (50% stem infection)	100 (stem infection in all)	No plates poured
3. *X. phaseoli* variant "M-associate"	0	0	0	0	0	12½	100 (scattering lesions on inoculated primary leaves)	100 (25% stem infection)	0[a] (plated July 3)
4. Virus-bearing plant juice plus *X. phaseoli* "M-associate"	0	0	0	+ (some infection on inoculated primary leaves)	+ (good infection on inoculated primary leaves)	100 (all inoculated leaves dead or dying)	100 (87½% stem infection)	100 (stem infection in all)	*X. phaseoli* masked in healthy looking trifoliate leaves; isolated June 22
5. Virus-bearing plant juice plus *X. phaseoli* variant "M-associate"	0	0	0	0	0	100 (scattering lesions on inoculated primary leaves)	100 (scattering lesions on inoculated primary leaves)	100 (25% stem infection)	*X. phaseoli* var. reverted to "typical" *X. phaseoli;* isolated from inoculated primary leaf, May 25

[a] Plated from dry bacterial ooze 65 days after inoculation; *X. phaseoli* variant probably dead therein.

Stringless Green Refugee (*Table 1*). Both *Xanthomonas phaseoli* "M-associate" and the virus-bearing plant juice plus the same (inocula 2 and 4) produced typical bacterial lesions on all the inoculated primary leaves. None were visible on the trifoliate leaves. On the other hand, the variant failed to produce visible bacterial infection whether used alone or with the virus (inocula 3 and 5). Nor did the virus-bearing plant juice alone produce bacterial lesions. This fact indicated that in this case no "typical" *Xanthomonas phaseoli* was masked in the inoculum.

Corbett Refugee (*Table 2*). The mosaic-immune variety was more severely infected by the bacteria than Stringless Green Refugee. In 4 of the 5 sets of inoculations (inocula 2 to 5), bacterial lesions were produced on the inoculated leaves and secondary infections appeared on the stems. Visible bacterial infection never appeared on the trifoliate leaves. Inoculations with the virus-bearing plant juice alone (inoculum 1) produced no sign of bacterial infection. *Xanthomonas phaseoli* variant "M-associate," both alone and with the virus-bearing plant juice (inocula 3 and 5), was, however, more mildly infectious than the two inocula containing "typical" *X. phaseoli* "M-associate" (inocula 2 and 4). It produced but scattering leaf lesions and much less stem infection.

No bacterial infection appeared on the checks of either variety.

Under the conditions of this experiment, the mosaic virus *Marmor phaseoli* was not inactivated by *Xanthomonas phaseoli* "M-associate," and *vice versa*.

The mosaic virus was not inactivated by *Xanthomonas phaseoli* variant "M-associate." The variant produced no bacterial symptoms on Stringless Green Refugee either alone or in combination with mosaic infected juice.[5] It did so on mosaic-immune Corbett Refugee, both alone and in association with the virus-bearing plant juice.

Reisolation of "Typical" Xanthomonas phaseoli

Wishing to watch the undisturbed development of these inoculated plants as long as possible, attempts at reisolation were too long deferred for best results, as it is often impossible to obtain "typical" *Xanthomonas phaseoli* from old leaf infections. However, "typical" *Xanthomonas phaseoli* was found *masked* in the trifoliate leaves of both varieties—in Stringless Green Refugee in leaves showing typical mosaic, in Corbett Refugee in apparently perfectly sound leaves. In both cases the inoculum had been the mixture of virus-bearing plant juice and *X. phaseoli* "M-associate" (inoculum 4) (Tables 1 and 2).

In order to isolate the masked bacteria from such trifoliate leaves, it is necessary to use a great deal more material than is customary in pouring plates from visible bacterial lesions. Judging from the number of colonies appearing in very heavily sown plates, the "typical" *Xanthomonas phaseoli* is present in comparatively very small numbers, at least in a form capable of cultivation on pH 7.0 beef-infusion agar.

[5] Compare inoculations 2 months later (Table 4).

"Typical" *Xanthomonas phaseoli* was also obtained from water-soaked spots on the inoculated primary leaves of Corbett Refugee inoculated with the virus-bearing plant juice plus *X. phaseoli* variant "M-associate" (inoculum 5). These leaves showed bacterial lesions typical of *X. phaseoli.* Under the conditions of this experiment, the *X. phaseoli* variant "M-associate" had reverted in the host to what has long been considered normal or typical *X. phaseoli.* (See Table 2.)

Isolation of S[6] Opaque White Colonies

Of special interest to the writer was the occurrence of smooth, shining opaque white colonies in the plates from all five sets of inoculations on Stringless Green Refugee—even from the set inoculated with the virus-bearing plant juice alone. (See Table 3.)

These colonies were similar in appearance to those now and then encountered in isolation plates of *Xanthomonas phaseoli* in years past. They also resembled colonies of one of the forms appearing later in plates from serial passages of infected juice containing both *X. phaseoli* and the virus.

In the early greenhouse experiments under consideration they came up in considerable numbers in platings from the sets inoculated with the 3 infective agents alone (inocula 1 to 3). They occurred in smaller numbers in plates from the 2 sets inoculated with a combination of the virus-bearing plant juice and the bacteria (inocula 4 and 5).

No such colonies came up in the plates poured from 3 of the 5 sets of the inoculated mosaic-immune Corbett Refugee.[7] No plates were poured from the 2 sets of this variety inoculated with virus-bearing plant juice (inoculum 1) or with *X. phaseoli* "M-associate" (inoculum 2).

This white form played a prominent rôle in dissociation studies, to appear in another paper. As had long been suspected by the writer, it was shown to be a mildly pathogenic variant of *Xanthomonas phaseoli.*

FIRST SCREENED-HOUSE EXPERIMENTS

The April experiments, described above, were repeated in the ensuing June and July. As hothouse temperatures run very high in summer, the plants were grown out-of-doors but under a 16-mesh[8] screened shelter for protection from the Mexican bean beetles. This of course reduced the light considerably and lowered the temperature somewhat. Nevertheless good infection resulted from both the virus-bearing infected juice and *Xanthomonas phaseoli* (Table 4).

In addition to the 2 isolates that had appeared to be mosaic-virus carriers in the previous experiment (Table 1), *Xanthomonas phaseoli* from a third source was tested. This isolate had no history of an association with the virus. It had been obtained from spotted Lima-bean pods from Cuba

[6] Smooth and shining.

[7] In later experiments, however, S opaque white colonies came up in isolation plates from Corbett Refugee inoculated with virus-bearing plant juice plus another strain of *Xanthomonas phaseoli.* No typical *X. phaseoli* was present.

[8] Replaced by 8-mesh after the first summer.

TABLE 3.—*S opaque white colonies isolated from inoculations of April 26, on Stringless Green Refugee bean plants*

Inoculum	S opaque white colonies	Comments	Mosaic	Typical *Xanthomonas phaseoli* infection
			Per cent	*Per cent*
1. Virus-bearing plant juice	+	Isolated June 6 and 14 from mosaic-virus-infected trifoliate leaves, stems and pedicels	75	0
2. *Xanthomonas phaseoli* "M-associate"	+	Isolated June 6 from mosaic-virus-infected trifoliate leaves	75	100
3. *X. phaseoli* variant "M-associate"	+	Isolated June 6 from mosaic-virus-infected trifoliate leaves	75	0
4. Virus-bearing plant juice plus *X. phaseoli* "M-associate"	+	Associated with "typical" *Xanthomonas phaseoli* masked in mosaic-virus-infected trifoliate leaves; isolated June 6	75	100
5. Virus-bearing plant juice plus *X. phaseoli* variant "M-associate"	+	Isolated June 6 from mosaic-virus-infected trifoliate leaves	100	0

and was designated *X. phaseoli* Cuba Strain. It had been isolated about 6 months earlier and kept in stock cultures on steamed potato cylinders in the refrigerator.

No other isolates of *Xanthomonas phaseoli* were available for testing in the screened house at this time.

Inoculations with the Bacteria Alone

Because of delays in the construction of the screened house, this inoculation experiment was about 2 months later than the greenhouse experiments. By this time both *Xanthomonas phaseoli* "M-associate" and *X. phaseoli* variant "M-associate" no longer appeared to carry the virus.[9] Each isolate caused bacterial infection on each bean variety.

Of the two "M-associates," *Xanthomonas phaseoli* variant was very much the less virulent (Table 4, No. 4). It is of considerable interest to note that when seemingly freed of its mosaic virus, the weakly parasitic variant was able to produce bacterial lesions on Stringless Green Refugee. This had not been the case when it appeared to be carrying the virus, although it had been able to do so on the mosaic-immune Corbett Refugee (Tables 1 and 2).

Xanthomonas phaseoli Cuba Strain (Table 4, No. 3) produced 100 per cent bacterial infection in both bean varieties, but no mosaic. This isolation had been growing on potato cylinders 6.4 months when tested.

Inoculations with the Virus-bearing Plant Juice Alone

The mosaic-virus-bearing plant juice alone produced 75 per cent typical mosaic on Stringless Green Refugee in 14 days, and none on Corbett Refugee (Table 4, No. 1). There was no evidence of bacterial infection on either variety, indicating that there had been no masked "typical" *Xanthomonas phaseoli* in the virus inoculum.

Tests of the juice of Corbett Refugee trifoliate leaves 20 days after rubbing the primary leaves with virus-bearing plant juice showed that the virus had not been able to maintain its existence in this mosaic-immune variety. Juice of the Stringless Green Refugee inoculated at the same time transmitted the virus readily.

Inoculations with Combined Bacteria and Virus-bearing Plant Juice

Of the two combined inocula, only that containing the weaker bacterial parasite, *Xanthomonas phaseoli* variant "M-associate" (Table 4, No. 6), produced any mosaic on the very susceptible Stringless Green Refugee. The reason for this is not clear. Is it because this variant with only mild pathogenicity offered less competition to the mosaic virus?

[9] These two isolates had been growing 3 and 2 months, respectively, on steamed potato cylinders which for this organism constitute an excellent culture medium if not over-cooked and if supplied with sufficient water to cover about half the cylinder. The cultures had been stored in the refrigerator after good growth had taken place. Young transfers on potato cylinders were used as inoculum.

TABLE 4.—*Inoculations in screened house on Stringless Green Refugee and Corbett Refugee bean plants*

Date of inoculation	Results on mosaic-susceptible Stringless Green Refugee								Results on mosaic-immune Corbett Refugee	
	Inoculum	Mosaic					Visible bacterial infection	Isolation	Mosaic infection	Visible bacterial infection
		6 days	8 days	9 days	12 days	14 days				
		No.	*No.*	*No.*	*No.*	*No.—%*	*Per cent*		*Per cent*	*Per cent*
1. June 18	Virus-bearing plant juice	0/8	0/8	0/8	No record	6/8—75	0		0/8	0
2. June 20	*Xanthomonas phaseoli* "M-associate"[a]	0/5	0/5	0/5	0/5	0/5—0	100 (severe)		0/8	100 (severe)
3. June 20	*X. phaseoli* Cuba Strain	0/7	0/7	0/7	0/7	0/7—0	100 (good)		0/7	100 (good)
4. June 24	*X. phaseoli* variant "M associate"[a]	0/8	0/8	0/8	0/8	0/8—0	37.5 (mild)		0/7	28.6 (mild)
5. July 3	Virus-bearing plant juice plus *X. phaseoli* "M-associate"	0/7	0/7	No record	0/7	0/7—0	100 (severe)		0/7	100 (severe)
6. July 3	Virus-bearing plant juice plus *X. phaseoli* variant "M-associate"	0/7	0/7	No record	2/7	2/7—28.6	No external symptoms; masked infection	"Typical" *Xanthomonas phaseoli* isolated Aug. 6; masked in mosaic-infected trifoliate leaf; reversion of *X. phaseoli* variant	0/8	No visible symptoms; probably was masked infection as Corbett had earlier proved (Tables 1 and 2) more susceptible in the greenhouse to weak variant than was Stringless Green Refugee

[a] Compare table 1.

As will be noted in table 4, the inoculations with the combination inocula containing the very virulent *Xanthomonas phaseoli* "M-associate" on the one hand and the mildly pathogenic variant on the other (Nos. 5 and 6) were made on the same day and hence under similar conditions. The source of mosaic virus was also the same, yet the combination inoculum containing the variant produced 28.6 per cent mosaic in 12 days, the other none.

Both (5 and 6) produced bacterial infection. As in the April experiment, *Xanthomonas phaseoli* variant reverted to "typical" *X. phaseoli* in the host (Tables 2 and 4). The 50 uninoculated plants remained healthy.

S Opaque White Colonies

S opaque white colonies like those recorded in the first inoculation experiment (Table 3) came up in considerable numbers in plates from month-old inoculations on Corbett Refugee. The inoculum in this case was *Xanthomonas phaseoli* Cuba + mosaic virus. The plants had shown 100 per cent "typical" *X. phaseoli* infection. No "typical" *X. phaseoli* appeared in these plates, although they were poured from a characteristic *X. phaseoli* lesion. On the other hand, the "typical" *X. phaseoli* was isolated the same day from Stringless Green Refugee, which had been inoculated at the same time and with the same inoculum. In this set of plates no S opaque white colonies appeared. Both platings were from old lesions, and long experience in isolating this organism has shown that the results of plating from dried tissues are extremely variable.

This set was not grouped with those in table 4 because, unlike sets 5 and 6, therein, a sterile distilled water suspension of the bacterium had been added to the plant juice containing the virus, thus diluting it considerably. No mosaic was produced.

TESTS OF ADDITIONAL ISOLATES OF XANTHOMONAS PHASEOLI WITH NO HISTORY OF ASSOCIATION WITH THE MOSAIC VIRUS

On November 16, the writer tested on Stringless Green Refugee and Corbett Refugee 2 recently obtained isolates of *Xanthomonas phaseoli,* unassociated with mosaic, viz.:

1. *Xanthomonas phaseoli,* "Barclay" strain, isolated in July from young infections on leaves of bush Lima bean grown at Barclay, Maryland (3.6 months on steamed potato cylinders).

2. *Xanthomonas phaseoli,* "Asgrow" strain, isolated in October from the defoliated tip of an Asgrow Stringless Green Pod plant from a test plot at Beltsville, Md. (5 weeks on steamed potato cylinders). The plants in the plot had been grown from seed stock used in a Maryland field where about 50 per cent of the plants had been reduced to "sticks" through defoliation.

3. For comparison, an "M-associate" isolate obtained in August from a trifoliate leaf with typical mosaic symptoms but no bacterial lesions.[10] It

[10] From screened-house inoculations of July 3, with the weakly parasitic *Xanthomonas phaseoli* variant "M-associate" + virus-infected juice (Table 4, No. 6).

had been growing 3.4 months on steamed potato cylinders and was a reversion of the original yellow "M-associate" variant to "typical" *X. phaseoli.*

In addition to the pure culture inoculations with isolates 1 to 3, three sets of plants were tested with these isolates plus virus-bearing plant juice, and a fourth set with the virus-bearing plant juice alone. The juice of typical mosaic-infected trifoliate leaves was used as inoculum, from plants grown from virus-infected seed.

The following results were obtained:

1. All 3 bacterial isolates caused 100 per cent severe bacterial infection; none produced mosaic. The "M-associate" isolate (No. 3) apparently had not carried the virus for 3.4 months in its stock cultures.

2. The virus-bearing plant juice alone produced 60 per cent mosaic in 17 days. There was no bacterial infection, which indicated that the inoculum had contained no masked *Xanthomonas phaseoli.*

3. Three sets inoculated with a combination of the virus with each of the 3 isolates, respectively, showed good bacterial infection but no mosaic. The lack of virus infection in this case was puzzling, especially in view of the 60 per cent mosaic produced by the virus-bearing plant juice alone without the use of carborundum. Was it because these very virulent bacterial isolates had offered too much competition to the virus?

4. With its reversion in the host to a "typical" *Xanthomonas phaseoli* strain (isolate 3), the original yellow "M-associate" variant had greatly increased in virulence.

5. A few of the S opaque white colonies previously mentioned (Table 3) were associated with "typical" *Xanthomonas phaseoli* in isolation plates poured 13 days after the inoculation of Stringless Green Refugee with *X. phaseoli,* "Asgrow" strain (isolate 2).

TYPES OF BACTERIA ISOLATED FOLLOWING AN IN VIVO ASSOCIATION OF XANTHOMONAS PHASEOLI CUBA STRAIN[11] AND THE MOSAIC VIRUS, MARMOR PHASEOLI

From February and March inoculations on Stringless Green Refugee with *Xanthomonas phaseoli* Cuba strain plus the mosaic-virus-bearing plant juice a number of types of bacteria were isolated. The above sets of inoculations had resulted in 44 to 69½ per cent mosaic and a mild bacterial infection. In addition to "typical" *X. phaseoli,* the following isolates were obtained:

1. *Xanthomonas phaseoli* variant-2,[12] a yellow isolate from the petiole of a typical mosaic-infected trifoliate leaf of Stringless Green Refugee. This was isolated 29 days after rubbing the primary leaves with the mixed inoculum. "Typical" *X. phaseoli* was isolated on the same day from the blade of the same trifoliate leaf. It was masked therein. Like the original

[11] An isolate from Cuba with no history of association with virus.

[12] Resembling the original yellow variant used in the first experiments. This is the Pvar. used later in the serial passages of plant juice containing mosaic virus plus *Xanthomonas phaseoli* variant.

yellow variant when first isolated, *X. phaseoli* variant-2 produced no visible infection on Stringless Green Refugee leaves (Table 1). It produced infection on pods, however, and did not revert to the "typical" *X. phaseoli* therein. The original yellow variant had so reverted in the leaves of Corbett Refugee (Table 2) and, later, in Stringless Green Refugee (Table 4, No. 6).

There was no evidence that variant-2 carried the virus.

2. Smooth, shining white colonies. These were associated in the isolation plates with *Xanthomonas phaseoli* variant-2 above. They were non-pathogenic or mildly pathogenic.

3. S pink colonies also associated with *Xanthomonas phaseoli* variant-2; non-pathogenic.

4. Smooth, shining, round, opalescent, rather coarsely cross-hatched colonies that attained a diameter of 7–9 mm. in the most thinly sown plates; producing no trace of green fluorescence in pH 7.0 beef-infusion agar stabs; isolated from Stringless Green Refugee inoculated 18 days previously with the combined virus-bearing plant juice and *X. phaseoli* Cuba strain.

The plates were poured from trifoliate leaves with typical mosaic symptoms but no bacterial lesions. The colonies appeared in 2 days in considerable numbers (160 cir. in the most heavily sown plate). Four days later a second organism (yellow) appeared in much smaller numbers (25 in the most heavily sown plate (see No. 5 below)). Nothing resembling "typical" *Xanthomonas phaseoli* or the original yellow variant ever came up in these plates from the mosaic-infected trifoliate leaves, although the *X. phaseoli* Cuba strain used in the combination inoculum was a virulent strain and typical *X. phaseoli* lesions had appeared on the rubbed primary leaves in 4 days. The cross-hatched, opalescent colonies above described were extremely pathogenic to pods (prick inoculations), whence they were recoverable. They also produced infection of the stem and pulvinus but no visible infection on sprayed, pricked leaf blades of Stringless Green Refugee.

5. The yellow organism associated with the preceding in the isolation plates was, to all appearances, also pathogenic to pods (prick inoculations), but was never recovered therefrom (5 sets of plates from 3 infected pods). On the contrary the very pathogenic (to pods) organism with smooth, opalescent, cross-hatched colonies (see No. 4 above) came up in the plates from all three of these pods (in pure cultures from two of them). In the third pod it was associated with a green fluorescent organism as well, which was likewise pathogenic to pods (prick inoculations) and recoverable from the infected tissues.

This series of isolations of various bacterial types from plants inoculated with a strain of *Xanthomonas phaseoli* of known virulence combined with virus-bearing plant juice cannot be viewed as proof that variation resulted from the action of the virus upon the bacterium, but such occurrences under these circumstances are interesting and should be recorded as a part of these association studies. As previously stated S white colonies have been en-

countered now and then in isolation plates of *X. phaseoli* from bean plants showing no mosaic symptoms. The same is true, though very much more rarely, of pink colonies. The other forms have not been noted in isolations from mosaic-free plants.

TESTS OF XANTHOMONAS PHASEOLI AND X. PHASEOLI VARIANT-2 AFTER 6 TO 63 DAYS' GROWTH IN VITRO FOLLOWING AN IN VIVO ASSOCIATION WITH THE VIRUS LASTING 28 TO 53 DAYS

In the early experiments, cultures of *Xanthomonas phaseoli* and the yellow variant, both M-associates, appeared to carry the virus in a virulent form as long as 6 weeks. After 2 or more months in stock, the cultures seemingly lost their ability to produce mosaic. To what extent might number of days' growth *in vitro* and of association with the virus *in vivo* be factors determining whether or not the bacterium could be a carrier of the virus?

In an attempt to shed some light on this question, a series of inoculations was made with isolates with histories of 6 to 63 days *in vitro* after previous association for 28 to 53 days with the virus *in vivo*.

The inocula consisted of all isolates in stock that had a history of association with the virus *in vivo*, viz.:

Isolate 1. *Xanthomonas phaseoli* isolated from the blade of a typical mosaic-infected trifoliate leaf (from a plant inoculated with mosaic-virus-bearing plant juice plus *X. phaseoli* Cuba strain.

Isolates 2, 3 and 4. Descendants of Isolate 1, after 1 or 2 passages through the plant in company with the virus-bearing plant juice.

Isolate A. *Xanthomonas phaseoli* variant-2 isolated from the petiole of the same trifoliate leaf as Isolate 1 (plates poured the same day).

Isolates B and C. Descendants of Isolate A after subsequent passage through the plant in company with the virus-bearing plant juice. Young cultures on steamed potato cylinders were used. Details of the *in vitro* and *in vivo* history of the isolates follow:

Xanthomonas phaseoli:

Isolate 1. Tested after 7, 14, 16, 27, 36, 37 days *in vitro;* previously associated with the virus *in vivo* 29 days.

Isolate 2. Tested after 27, 35, 47 days *in vitro;* previously associated with the virus *in vivo* 28 days.

Isolate 3. Tested after 63 days *in vitro;* previously associated with the virus *in vivo* 28 days.

Isolate 4. Tested after 6 days *in vitro;* previously associated with the virus *in vivo* 49 days.

Xanthomonas phaseoli variant-2:

Isolate A. Tested after 15 and 16 days *in vitro;* previously associated with the virus *in vivo* 29 days.

Isolate B. Tested after 12 days *in vitro;* previously associated with the virus 53 days *in vivo*.

Isolate C. Tested after 17 days *in vitro;* previously associated with the virus *in vivo* 53 days.

No mosaic was produced in any of the 15 sets of leaf-inoculations (all made on Stringless Green Refugee). In other words there was no evidence that in these cases the bacterium was carrying the virus. When these same isolates were combined with mosaic-virus-bearing plant juice, mosaic was always produced.

There was, furthermore, no indication that association with the virus *in vivo* for 28 to 53 days had lessened the ability of "typical" *Xanthomonas phaseoli* to produce bacterial lesions. *X. phaseoli* variant-2, on the other hand, produced no bacterial lesions on leaves. Other tests proved it to be infectious to pods after 9 and 55 days *in vitro,* following 29 days' association with the virus *in vivo.*

By this time it was apparent that mere association *per se* was not the sole answer to the surprising results obtained in the first experiment. At that time inoculation with seemingly pure cultures of *Xanthomonas phaseoli* and *X. phaseoli* variant, each of which had been associated with the mosaic virus *in vivo* (Table 1), had produced 75 per cent typical mosaic on plants grown from seed collected by W. J. Zaumeyer, from healthy plants in his Greeley, Colorado, breeding plots. There had been no sign of seed infection in the 50 uninoculated checks.

At this point the problem was attacked from a new angle.

PART II

SERIAL PASSAGES OF ASSOCIATED INFECTIVE AGENTS

The association of the mosaic virus and *Xanthomonas phaseoli* occurs under natural conditions in the seed. The two original bacterial carriers of the mosaic virus had such an origin. This association may have been going on indefinitely for many generations. These facts led to the conclusion that it would be desirable to know what would be the effect of continual uninterrupted association of the virus and the bacterium in the bean plant without any intervention of culture media. Accordingly, after the 1½ years of preliminary work reported in Part I, there were inaugurated 2 sets of serial passages of juice containing the two infective agents. Stringless Green Refugee, susceptible to both pathogens, was used as the host plant.

For the sake of brevity the 2 series will be designated (M + P) and (M + Pvar) with superscripts indicating the number of the serial passage.

The work herein described covers 50 serial passages and a period of 3¼ years. The writer will not attempt to give the details of the individual passages, but will point out the facts that have stood out as high lights in this period of uninterrupted association *in vivo* of the infective agents.



MATERIALS AND METHODS

Inocula

The inocula used to inaugurate the serial passages were:

1. (M + P): Juice from typical mosaic-infected trifoliate leaves of

Stringless Green Refugee beans. The plants had been inoculated 3 weeks before[13] with *Xanthomonas phaseoli* + mosaic-virus-bearing plant juice.

2. (M + Pvar): Juice from similar trifoliate leaves from plants inoculated 3½ weeks before[14] with *X. phaseoli* variant-2 + mosaic-virus-bearing plant juice.

Both the *Xanthomonas phaseoli* and the *X. phaseoli* variant-2 which were contained in juices 1 and 2 had descended via a short series of plant passages and re-isolations from the same typical mosaic-infected trifoliate leaf.[15] *X. phaseoli* had been isolated from the leafblade and *X. phaseoli* variant-2 from the petiole on the same date.

Methods of Inoculation

The methods pursued during these serial passages were similar to those previously described with the exception that in this case no cultures of the bacteria were used. The only inoculum was juice containing the virus and one of the bacterial isolates. For each serial passage juice from plants of the preceding one was used as inoculum.

As a rule the passages were made at 3-week intervals. Carborundum was found unnecessary and was discontinued after the fifth serial passage. There was an average of 20.9 inoculated plants in each set.

Numerous uninoculated checks were held throughout the period of investigation and the total incidence of mosaic among them was less than 0.4 per cent. There was still less bacterial infection.

Although the possibility of occasional seed infection cannot be ruled out absolutely, there is overwhelming evidence, as seen above, of the disease-free character of the seed stock of Stringless Green Refugee as a whole.

HIGH LIGHTS IN SERIAL PASSAGES

I. The Virus

1. *Domination of the Virus:* From the beginning domination of the virus has been observed, and has become increasingly more apparent as the serial passages increased in number.

2. *Increasing Percentage of Mosaic Infection with Increased Number of Serial Passages.* (M + P): From an average of 60.7 per cent mosaic in the first 10 serial passages the amount of virus infection rose to 100 per cent throughout the fifth decad. The maximum, minimum, and average per cent of virus infection in the sets of the 5 decads are shown in table 5 together with the numbers of sets showing the indicated percentages and the numbers of inoculated plants on which the percentages are based.

(M + Pvar): The rise in the amount of infection in this series was only slightly less spectacular than was that in (M + P). Details of maximum, minimum, and average infection are shown in table 6.

[13] Showed 48 per cent mosaic and considerable *Xanthomonas phaseoli* infection.
[14] Showed 100 per cent mosaic and little if any sign of bacterial infection.
[15] From a plant inoculated with *X. phaseoli* Cuba Strain + mosaic-virus-bearing plant juice.

TABLE 5.—*Serial passages of (M + P)*

(M + P) series	Maximum virus infection	Minimum virus infection	Average virus infection
Decad	*Per cent*	*Per cent*	*Per cent*
1st (199 plants)	100 (3 sets—32/32)	14.3 (1 set—2/14)	60.7
2nd (201 plants)	100 (2 sets—43/43)	53.3 (1 set—8/15)	78.6
3rd (193 plants)	100 (2 sets—38/38)	31 (1 set—5/16)	63.0
4th (218 plants)	100 (3 sets—71/71)	80 (2 sets—24/30)	92.3
5th (233 plants)	100 (10 sets—233/233)	.....	100.0

3. *Sudden Onset of Ultra-severe Mosaic Infection:* In the beginning, and for 37 succeeding serial passages, the mosaic infection in both series was, on the whole, mild. Only moderate stunting and a few typically infected (curled and mottled) trifoliate leaves were produced. With the 38th serial passage (both series),[16] however, there was in all the inoculated plants a sudden onset of an ultra-severe form of mosaic. This continued with all succeeding passages throughout the 50 here described. Possible reasons for this sudden change in degree of virulence will be discussed later.

In the ultra-severe mosaic there was extreme dwarfing and bunching of tiny shoots (Fig. 1). The majority of the trifoliate leaves were minute and more or less filiform; many of them consisted of little more than a midrib. The primary leaves and the larger trifoliate leaves, if any, showed striking discoloration and very conspicuous veining. This was unlike the typical mosaic mottling, and indicated extreme disturbance of the physiological functioning of the plant. Only an occasional plant produced pods or even blossoms. At this point the writer found it necessary to use whole tops of plants instead of selected trifoliate leaves as inoculum.

TABLE 6.—*Serial passages of (M + Pvar)*

(M + Pvar) series	Maximum virus infection	Minimum virus infection	Average virus infection
Decad	*Per cent*	*Per cent*	*Per cent*
1st (203 plants)	100 (1 set—16/16)	28.6 (1 set—2/7)	62.1
2nd (199 plants)	91 (1 set—21/23)	15.4 (1 set—4/26)	53.8
3rd (171 plants)	100 (2 sets—26/26)	21.7 (1 set—5/23)	67.0
4th (219 plants)	100 (3 sets—61/61)	52 (1 set—13/25)	77.4
5th (257 plants)	100 (9 sets—224/224)	84.8 (1 set—28/33)	98.5

[16] It is interesting to note that, since for various reasons the (M + Pvar) serial passages fell 2 behind those of (M + P), the 38th serial passages of the 2 sets did not occur on the same date. That of (M + Pvar) was made on January 16: that of (M + P) on the 28th of the preceding November, or 2 years 4½ months and 2 years 3 months, respectively, after the inception of the serial passages. Light conditions, according to the Weather Bureau, were approximately the same on the 2 dates.

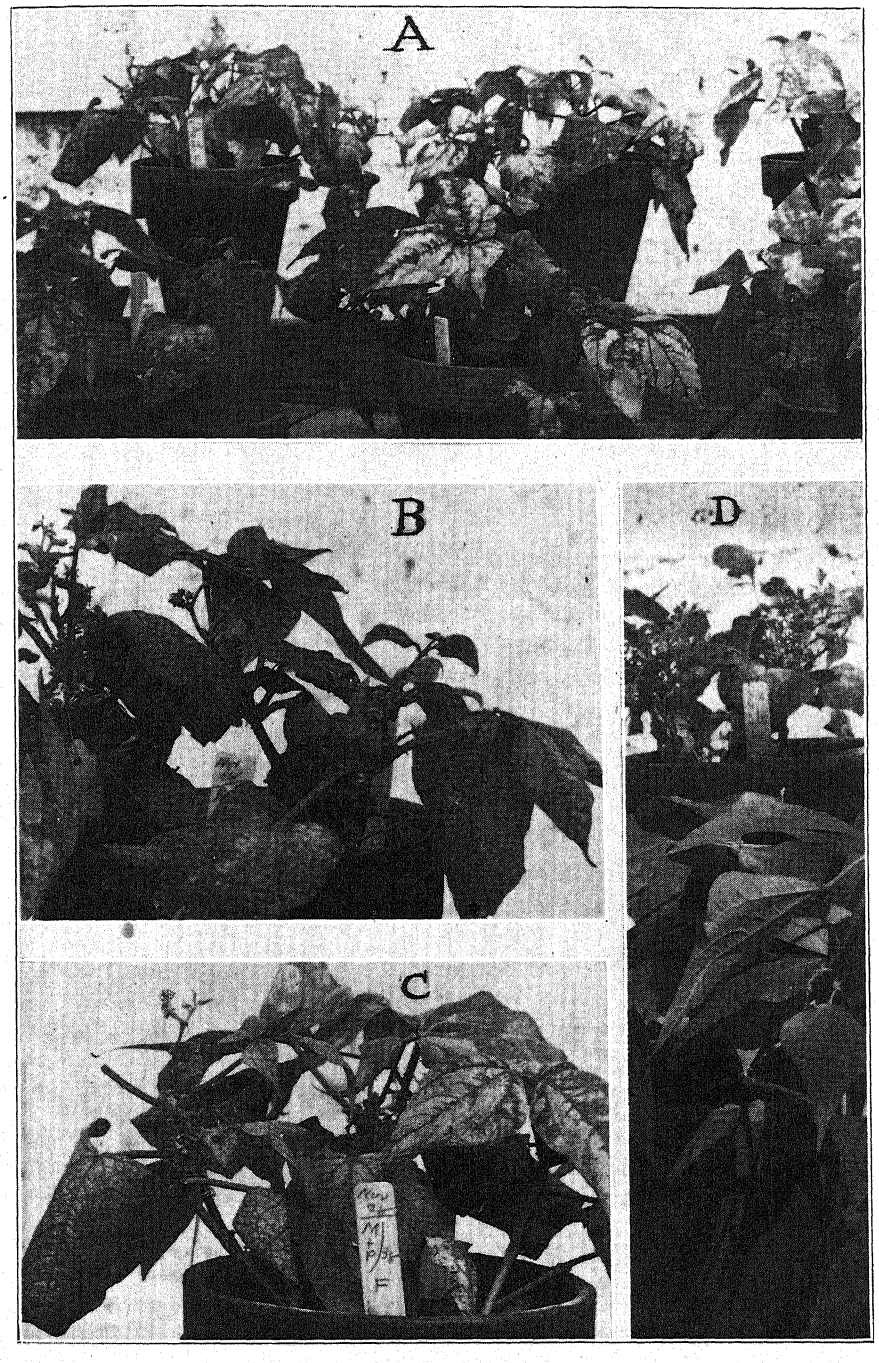


FIG. 1. A. Stringless Green Refugee bean plants 6 weeks after inoculation (when 13 days old) with the 37th serial passage of mosaic-virus-bearing plant juice plus *Xanthomonas phaseoli*, $(M+P)^{38}$, showing extreme dwarfing, bunching, and discoloration; no pods or blossoms. B and C. Details of above. D. Plants (in background) similarly inoculated with the 39th serial passage of virus and bacterium, $(M+P)^{40}$, 7 weeks after inoculation; portion of uninoculated check below.

II. The Bacterium

The following observations apply to both series, (M + P) and (M + Pvar).

1. *Comparatively Inconspicuous Bacterial Symptoms.* The bacteria in association with the mosaic virus in these serial passages have always appeared to play a subordinate rôle. They have, as a rule, produced rather inconspicuous symptoms confined to the inoculated primary leaves. These in most cases might be easily overlooked. On the other hand, there were occasional severe symptoms caused by bacterial infection.

2. *Sudden Disappearance of the Bacterial Symptoms.* All bacterial symptoms suddenly disappeared after 25½ months of uninterrupted association of the infective agents *in vivo* ((M + P)[36] and (M + Pvar)[34]) and shortly before the onset of the ultra-severe form of the mosaic. After 10 successive passages, covering a period of 7 5/6 months, they reappeared on the inoculated primary leaves.

3. *Recovery of Typical Form of Xanthomonas phaseoli from Serial Passages.* Even though bacterial symptoms were only rarely more than barely noticeable, it was usually fairly easy, before their complete disappearance, to recover the typical form of *X. phaseoli* from those trifoliate leaves showing mosaic but no bacterial symptoms.

Such isolates were always virulent when tested. Extremely virulent isolates of *Xanthomonas phaseoli* were obtained, in fact, from the second serial passage (both series) preceding[17] the one in which bacterial symptoms were wholly lacking. These isolates were entirely "typical" in appearance in the plates and on steamed potato cylinders. *X. phaseoli* variant had reverted long since to the "normal" form. The above *X. phaseoli* isolate was one of those later used in dissociation experiments that furnished a spectacular demonstration of the changes seemingly undergone by the bacterium in its long, uninterrupted sojourn in the plant in association with the virus. This will be discussed later.

Typical *Xanthomonas phaseoli* also was isolated from both (M + P)[35] and (M + Pvar)[33], the sets immediately preceding the disappearance of all signs of bacterial infection. These isolates were not tested for pathogenicity.

Plants inoculated with 4 out of 20 isolates tested showed, in addition to bacterial infection, a small amount of mosaic, as follows: Isolate 1: from (M + P)[1]: 13.3% mosaic—2/15; Isolate 2: from (M + P)[9]: 6.9% mosaic—2/29; Isolate 3: from (M + Pvar)[24]: 13.6% mosaic—3/22; Isolate 4: from (M + P)[26]: 4.8% mosaic—1/21. The approximately 200 plants used as checks in testing these 20 isolates showed but 1 case of mosaic, or 0.5% (1/200).

4. *Dissociating S Yellow Xanthomonas phaseoli Colonies.* Some of the plates poured, both before and after the onset of the ultra-severe mosaic, contained dissociating colonies of the "typical" S yellow form of *Xanthomonas phaseoli.* Some of these colonies were very striking in appear-

[17] (M + P)[34] and (M + Pvar)[32]. These had shown 92 per cent and 95 per cent mosaic, respectively, combined with 100 per cent good but not severe bacterial infection.

ance (Fig. 2). They ranged from S yellow colonies with S white marginal fan-shaped outthrusts to those in which all the periphery was S white, while the S yellow was reduced to a central portion resembling a 3- or 4-petalled flower. Sometimes there were islands of S white in the "typical" S yellow growth.

5. *Isolation of Atypical Forms.* Following the disappearance of the bacterial symptoms, the writer was unable to isolate "typical" *Xanthomonas*

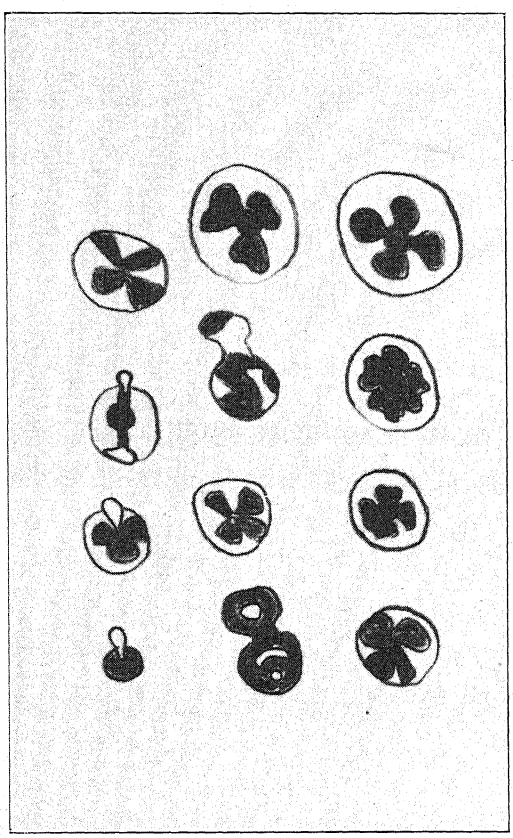


FIG. 2. Diagrammatic representation of dissociating colonies of *Xanthomonas phaseoli* in pH 7.0 beef-infusion-agar plates from serial passages of (M+P) and (M+Pvar)—bean plant juice containing the mosaic virus plus the bacterium or its variant. The shaded portion represents what remains of the "typical" S yellow; the unshaded area, the mildly pathogenic white variant.

phaseoli until the symptoms reappeared. On the other hand, the number of S opaque white colonies steadily increased. During the first year they were present in only 15 per cent of the serial passages from which platings were made; in the third year, in 68.8 per cent. In the early part of the third year, the bacterial symptoms had disappeared and the sudden onset of ultra-severe mosaic followed shortly thereafter.

An S pink form has occurred much less frequently and usually in smaller numbers, but has been found now and then ever since the serial passages

began. When it appears it is in most cases a minority group associated with "typical" *Xanthomonas phaseoli* or the S opaque white colonies.

Pin-point colonies were now and then encountered in large numbers. They sometimes far outnumbered those of the "typical" *Xanthomonas phaseoli*. There were also colorless ± R[18] colonies with filamentous outgrowths of the Medusa-head type. Microscopic examination of the latter showed that they were made up of filaments.

At this point it seemed apparent that the bacterium had been undergoing some change during its association with the virus *in vivo*. In order to shed some light on this phase of the interaction of the two infective agents, dissociation experiments were undertaken. The results of those studies will be reported in a second paper.

DISCUSSION

1. Did the bacterium carry the virus at any time during the experiment? There were more negative than positive results. The fact, however, that it appeared to carry the virus in the first experiment and to a lesser degree in the case of 4 isolates from the serial passages of (M + P) and (M + Pvar) is thought-provoking and worthy of record. In this connection it is of interest to note the order in which the mosaic symptoms appeared in relation to the location of the pots in the following two cases in the first greenhouse experiment:

A. In inoculations with *Xanthomonas phaseoli* M-associate April 26 (Table 1, inoculum 2) mosaic appeared as follows:

Pots in order of inoculation	6 days	8 days	12 days	20 days	25 days
Pot 1	0	0	0	0	1/2
Pot 2	1/2	2/2	2/2	2/2	2/2
Pot 3	0	?	?	?	1/2
Pot 4	0	0	0	1/2	2/2

Note: Numerator indicates number of plants infected; denominator, the number inoculated.

From the above data the writer concludes:

a. If pots 1, 3 and 4, had had infected seed, mosaic would probably have appeared sooner.

b. If pot 2 had had infected seed, infection would probably have been carried by the inoculating pad to pots 3 and 4 in which mosaic would in all probability have shown up sooner.

B. In inoculations with *Xanthomonas phaseoli* variant (Table 1, inoculum 3) mosaic symptoms appeared as follows:

Pots in order of inoculation	6 days	8 days	12 days	20 days	25 days
Pot 1	1/2	2/2	2/2	2/2	2/2
Pot 2	0	?/2	?/2	?/2	?/2
Pot 3	1/2	1/2	2/2	2/2	2/2
Pot 4	1/2	1/2	2/2	2/2	2/2

Note: Numerator indicates number of plants infected; denominator, the number inoculated.

[18] Rough.

From this the writer concludes:

a. Pot 1 seemingly did not have seed infection as mosaic did not spread from it to pot 2.

b. Pots 3 and 4 may have contained infected seed, though the possibility seems remote in view of the facts that the 50 check plants showed no mosaic symptoms and no aphids or evidence of their work were observed at any time.

2. Were the sudden onset of the ultra-severe mosaic and its persistence due to the long *in vivo* association with the bacterium? This cannot be answered by yes or no. Another virus may have come into the picture or there may have been virus mutation.

Bean virus 4, which was discovered in Louisiana two years later and gives very mild symptoms on Stringless Green Refugee (38), was tested in later studies in combination with bean virus 1. It did not increase the virulence of the latter (unpublished work). No carborundum was used.

3. Did the virus influence the tendency of the bacterium to dissociate? The writer believes this to be the case, although this question cannot be definitely answered until a study has been made of serial passages in Stringless Green Refugee bean plants of "typical" *Xanthomonas phaseoli* alone to determine whether the host can produce such an effect. Comparative dissociation studies of an isolate from the 34th serial passage of (M + P) and of a "normal" strain of *Xanthomonas phaseoli* showed that changes had occurred in the bacterium during its 2 years' *in vivo* association with the virus, whatever may have been the cause (unpublished work).

This paper raises more questions than it answers. The writer feels that it is thought-provoking and submits it in the belief that it is worth while for an investigator to share with fellow workers observations that are sometimes puzzling. Later discoveries often explain results that were unintelligible in the past, and show them to be of outstanding importance. Such has been the history of the progress of science through the ages.

The pathologists are, in contra-distinction to some other groups of biologists, working with two living variables, the host and the parasite. In the case under consideration there are three, granted, for the sake of the argument, that the virus belongs to the realm of living things.

Till comparatively recent years the majority of bacteriologists have considered as contaminants such variants as they encountered and have made no study of their possible significance. Now, however, the days when a bacteriologist was considered a heretic if he questioned the fixity of species, are no longer with us. A certain amount of variation might now be considered the rule rather than the exception. There is variation even among the progeny of single cells! Mellon's (19) phrase "The polyphasic potencies of the bacterial cell" expresses very well the view of many present-day bacteriologists.

The virus is even more of an enigma. There is, as yet, no complete acceptance of any one theory as to its nature, although it has been demonstrated that several plant viruses can be obtained in purified form as crystalizable proteins of extremely high molecular weight.

Of the host we are much less ignorant but there still are many unsolved problems that confront the investigator of the host-parasite relationship.

With this apologia, the writer submits the following summary:

SUMMARY

These studies were undertaken for the purpose of determining (a) whether mutual or unilateral antagonism or stimulation resulted from the *in vivo* association of the two seed-borne infective agents, *Xanthomonas phaseoli* (E. F. Sm.) Dowson and the virus of the common bean mosaic *Marmor phaseoli* Holmes (bean virus 1); (b) the application of any such interaction to the problems of breeding for disease resistance.

The expressed juice of mosaic-infected trifoliate leaves of *Phaseolus vulgaris* L. used by an associate in testing for mosaic-resistance a bean hybrid (later known as U.S. No. 5 Refugee) produced widespread typical *Xanthomonas phaseoli* lesions on the rubbed primary leaves.

Virulent *Xanthomonas phaseoli* and a less pathogenic yellow variant of the same were found by the writer to be masked in trifoliate bean leaves showing only symptoms of typical common bean mosaic.

Seventy-five per cent typical mosaic developed in one experiment on Stringless Green Refugee inoculated with single-colony cultures of 2 bacterial isolates, viz., "typical" *Xanthomonas phaseoli* from the bacterial lesions on primary leaves (see No. 2 Table 1) and *X. phaseoli* variant masked in the trifoliate leaves (see No. 3 Table 1). The bacteria appeared to be carriers of the virus. The mosaic equalled in amount and was in no way distinguishable from that produced on plants inoculated with the virus alone. The "typical" *Xanthomonas phaseoli* produced 100 per cent bacterial infection on the same Stringless Green Refugee individuals and also on Corbett Refugee. On the same date the less pathogenic variant produced bacterial lesions on the mosaic-immune Corbett Refugee only.

The apparent ability to produce mosaic was retained by the "typical" *Xanthomonas phaseoli* for at least 6 weeks in stock cultures on steamed potato cylinders. About 2 weeks of this time the cultures were held at laboratory temperatures. According to all published records, 1 to 2 days is the limit of "longevity" of the common bean mosaic virus in expressed juice at room temperatures.

The mildly pathogenic yellow variant, after losing its seeming initial ability to produce mosaic, was able to cause bacterial symptoms on Stringless Green Refugee, not the case hitherto.

Serial passages of infected juice containing both infective agents showed the domination of the virus and a marked increase in the amount and severity of virus infection. With the 38th serial passage, 100 per cent extreme dwarfing and bunching was accompanied by the reduction of the majority of the trifoliate leaves to a minute filiform state consisting of little more than midribs. This ultra-severe form of the mosaic persisted throughout the remainder of the approximately 50 passages herein described. Striking

discoloration and conspicuous veining of the primary leaves, and of the large trifoliate leaves if there were any, indicated an extremely deranged metabolism. Only an occasional plant blossomed.

Bacterial symptoms in the serial passages were as a rule, comparatively inconspicuous. They abruptly disappeared shortly before the sudden onset of the ultra-severe form of the mosaic. They recurred on the inoculated primary leaves only after 10 serial passages.

Virulent isolates of seemingly "typical" *Xanthomonas phaseoli* were readily obtained from the serial passages as long as bacterial symptoms were manifest. After the disappearance of the symptoms, however, colonies of the S opaque white mildly pathogenic form were present in increasing numbers in the plates. The pink colonies also appeared from time to time, usually as a minority group. Later studies (unpublished work) showed that both the white and pink forms are variants of the virulent yellow *X. phaseoli.*

No typical *Xanthomonas phaseoli* isolates were again obtained until bacterial symptoms recurred on the inoculated primary leaves.

An occasional set of plates showed colonies of *X. phaseoli* which were spectacularly dissociating into S white and S yellow.

The changes occurring in the bacterium in the course of the serial passages were further demonstrated by dissociation studies to be reported in a later paper.

In brief the studies showed that:

1. *Xanthomonas phaseoli* may be masked in either mosaic-susceptible or mosaic-immune bean varieties. Mosaic-infected trifoliate leaves, used customarily as bean virus 1 inoculum, were not infrequently symptomless carriers of *Xanthomonas phaseoli.*

2. In one experiment bean-mosaic virus apparently persisted in cultures of *Xanthomonas phaseoli* on steamed potato for as long as 6 weeks and in those of *X. phaseoli* variant for 11 days. With the possible exception of 4 isolates from the serial passages, no evidence of such persistence of the virus in culture was detected in other more extensive trials.

3. In the long-continued *in vivo* association of the two seed-borne pathogens in serial transfers from bean plant to bean plant, there occurred a decrease in the pathogenicity of the bacterium and a more or less steady increase in that of the virus to the point of extreme intensity, and individual variation in mosaic-resistance was no longer manifest.

4. Variants of *Xanthomonas phaseoli* differing in colony type and in virulence appeared in reisolation plates from bean plants inoculated with a combination of the bacterium and the mosaic virus. It is suggested that the virus influenced the tendency of the bacterium to dissociate.

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INTRACELLULAR INCLUSIONS IN TOBACCO MOSAIC–INFECTED NICOTIANA GLUTINOSA AND ITS HYBRIDS[1]

MARK W. WOODS

(Accepted for publication January 26, 1944)

Tobacco mosaic virus (*Marmor tabaci* H.) induces the formation of both crystalline and amorphous inclusions in cells of suscepts, which develop systemic chlorotic symptoms following infection. Such inclusions have not hitherto been reported in suscepts such as *Nicotiana glutinosa* L., which respond necrotically to infection with this virus. Sheffield (4) was unable to find inclusions in the necrotic local lesions in infected *N. glutinosa.* Since the observations of Beale (2), Bawden and Sheffield (1), *et al.,* it has become increasingly evident that the crystalline inclusions characteristic of tobacco mosaic represent either virus alone, or a virus-host constituent complex composed largely of virus protein. The "X"-bodies of this disease likewise contain much virus protein (1). Because of the etiologic significance of these inclusions it is of interest to know definitely whether their formation can be induced in necrotic-type suscept species. The experiments reported here have proved that under certain environmental conditions hexagonal crystals and "X"-bodies, typical of tobacco mosaic, can form in mosaic-infected cells of *N. glutinosa* and certain of its necrotizing hybrids. Necrotic response to tobacco mosaic virus in this species is controlled by a single dominant gene (3).

Detached leaves of both *Nicotiana glutinosa* and an F_2 hybrid heterozygous for the necrotizing gene (*N. tabacum* var. Turkish × *N. glutinosa*) were employed in the tests. The upper surface of one half of each leaf was inoculated by rubbing with a freshly prepared extract of green-mottling tobacco mosaic virus (*Marmor tabaci* H.). The control half of each leaf was similarly rubbed with sterile water. The leaves were rinsed with tap water immediately after inoculation and cut transversely into 4 sectors. Three sectors of each leaf were held in water-saturated atmospheres in sealed glass containers (approx. 1 l. capacity) in absolute darkness at 27 to 28° C. Each of these sectors was exposed to a different gas mixture in the containers. Container (a) was filled with plain air; container (b) with 73 per cent of oxygen, 17 per cent of nitrogen and 10 per cent of carbon dioxide; container (c) was filled with 90 per cent of oxygen and 10 per cent of nitrogen. In each container the by-products of respiration were allowed to accumulate. The fourth leaf sector was held in darkness at the same temperature in an unsealed container, the lower half-inch of the leaf sector being immersed in tap water. Each experiment involved from 1 to 3 leaves. A typical experiment gave the following results:

Six days after inoculation the leaf sectors that had been maintained in the sealed container of plain air, and in 90 per cent of oxygen, had yellowed

[1] The research here reported was carried on while the writer was a member of the staff of the Maryland Agricultural Experiment Station.

noticeably. The yellowing was particularly pronounced in the tissues held at the high oxygen concentration. In both cases the lesioned areas were only partly necrotic, and, though yellowish, had retained more of their original green color than noninfected tissues.

The leaf sectors maintained in an atmosphere containing 10 per cent of carbon dioxide were still green at the end of the experiment. The lesioned areas, which were barely discernible, appeared to be slightly deeper green than the noninfected tissues. Each lesion was bordered by a faint water-soaked green necrotic ring. These visible lesions from sectors treated with 10 per cent of carbon dioxide were slightly smaller than those formed in plain air or 90 per cent oxygen. This appeared to be due to masking of infected zones surrounding the necrotic rings since typical intracellular inclusions were observed in cells outside of the latter areas. These experiments demonstrated that 10 per cent of carbon dioxide not only retarded breakdown of the chlorophyll in noninfected tissues, but that this protective action extended to infected cells as well. In the latter case the necrotizing action of the virus was also materially reduced.

Leaf sectors held in the open containers (low humidity) developed solid-necrotic lesions that rapidly dried out.

Cytological examination of infected and control tissues from each set of experimental conditions revealed distinct differences between treatments. Crystalline inclusions and "X"-bodies were never observed in lesioned areas of leaf sectors held in the un-sealed containers. Both types of inclusions were found in epidermal and mesophyll cells in lesions from leaves held in each of the sealed containers, being observed most frequently in material treated with 10 per cent of carbon dioxide. Apparently the combination of high humidity plus an increased concentration of carbon dioxide was the most favorable condition for development of both types of inclusions. The hexagonal crystals formed in *Nicotiana glutinosa* and its hybrid appeared as perfect hexagons in face view with striate rectangular cross sections, or as irregular aggregates of 2 or more crystals. They were essentially like those observed in systemic lesions in Turkish tobacco, except for a lack of stability in the cell. In mounts of *N. glutinosa* or hybrid tissue in tap water at 25 to 28° C., perfect hexagonal crystals were observed to become irregular in outline after 5 or more minutes and to go rapidly into solution. A foamy residue was left in the cytoplasm at the point previously occupied by the crystal. Crystals in mosaic-infected Turkish tobacco leaves have not been observed to break down under similar conditions, although application of ether, hexylresorcinol 1–1000 ("ST–37"), dilute alcohol and mineral acids will cause them to break down.

In all of the lesions examined the crystals and "X"-bodies were observed more frequently in a zone some 15 cells wide on either side of the necrotic areas than in the non-necrotic centers or further from the periphery. Amorphous masses somewhat resembling the crystals were sometimes observed in completely necrotic cells. When the content of the dead cells

had oxidized to a brown color these masses and the nucleus also were colored a deep-brown. The regular cytoplasmic streaming sometimes observed in cells containing both hexagonal crystals and "X"-bodies indicates that these cells in the lesioned areas of *N. glutinosa* were living. In one case a cell with streaming cytoplasm and a large "X"-body was immediately adjacent to a completely necrotic cell.

Previous studies have shown that virus multiplication is dependent upon the functioning of a CN-sensitive respiratory system (5). The factors of high humidity, increased carbon dioxide concentration and darkness (modified gas exchange through the stomata) might similarly interact to modify the development of tobacco-mosaic symptoms from a complete necrosis without formation of intracellular inclusions to a less severe breakdown of the tissues.

The formation of hexagonal crystals and "X"-bodies seems to be associated with this lessening of the necrotic action of the virus on the cells.

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ANATOMICAL EFFECTS OF OIL SPRAY INJURY IN GUAYULE SEEDLINGS

FREDRICK T. ADDICOTT

(Accepted for publication February 5, 1944)

Oil sprays have been found very efficient in the control of weeds in the nurseries and fields where guayule is under cultivation. During the period of the development of spraying methods injury to the plants frequently was

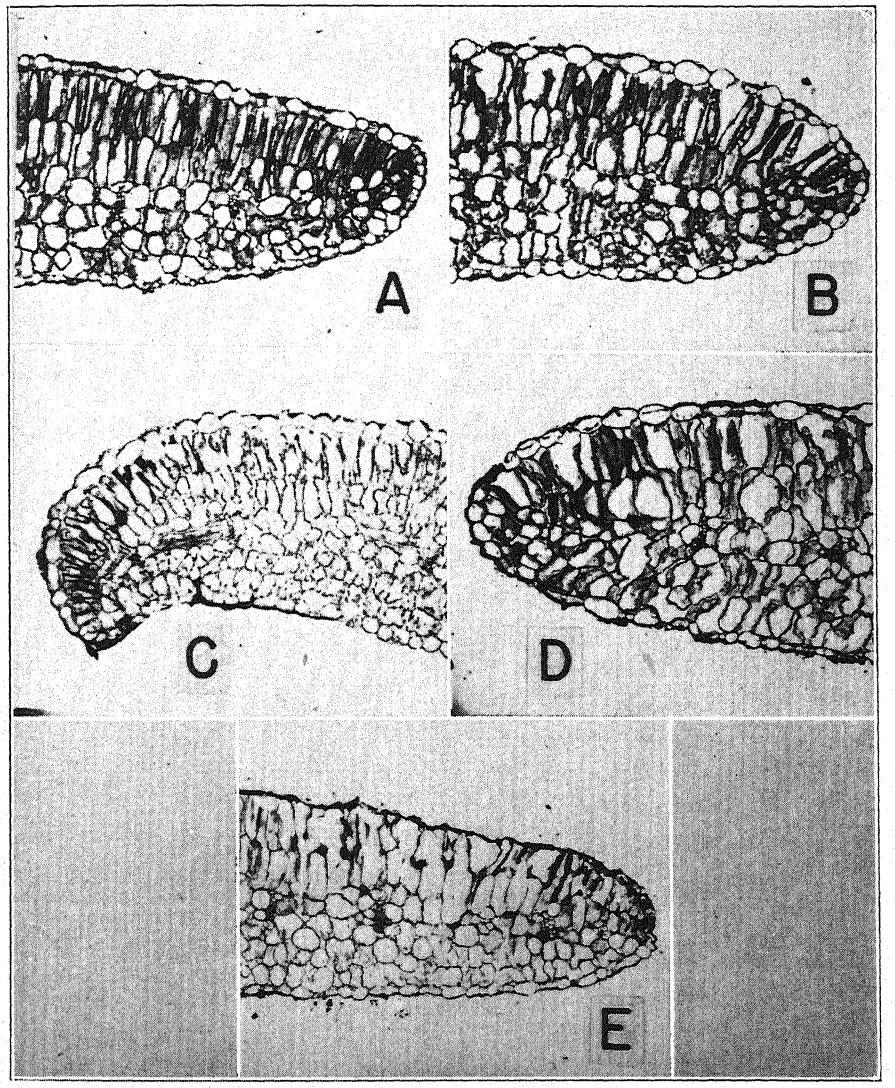


FIG. 1. Sections of oil-sprayed cotyledons. A. Uninjured cotyledon. B. 30 minutes after exposure to injurious amounts of oil spray. C. 2 hours after exposure. D. 24 hours after exposure. E. 48 hours after exposure. Magnification about 95 ×.

obtained. For the information of both research workers and those concerned with the production of guayule, an investigation of the anatomy of the injury was undertaken. It appears that these results may be of general interest also to pathologists.

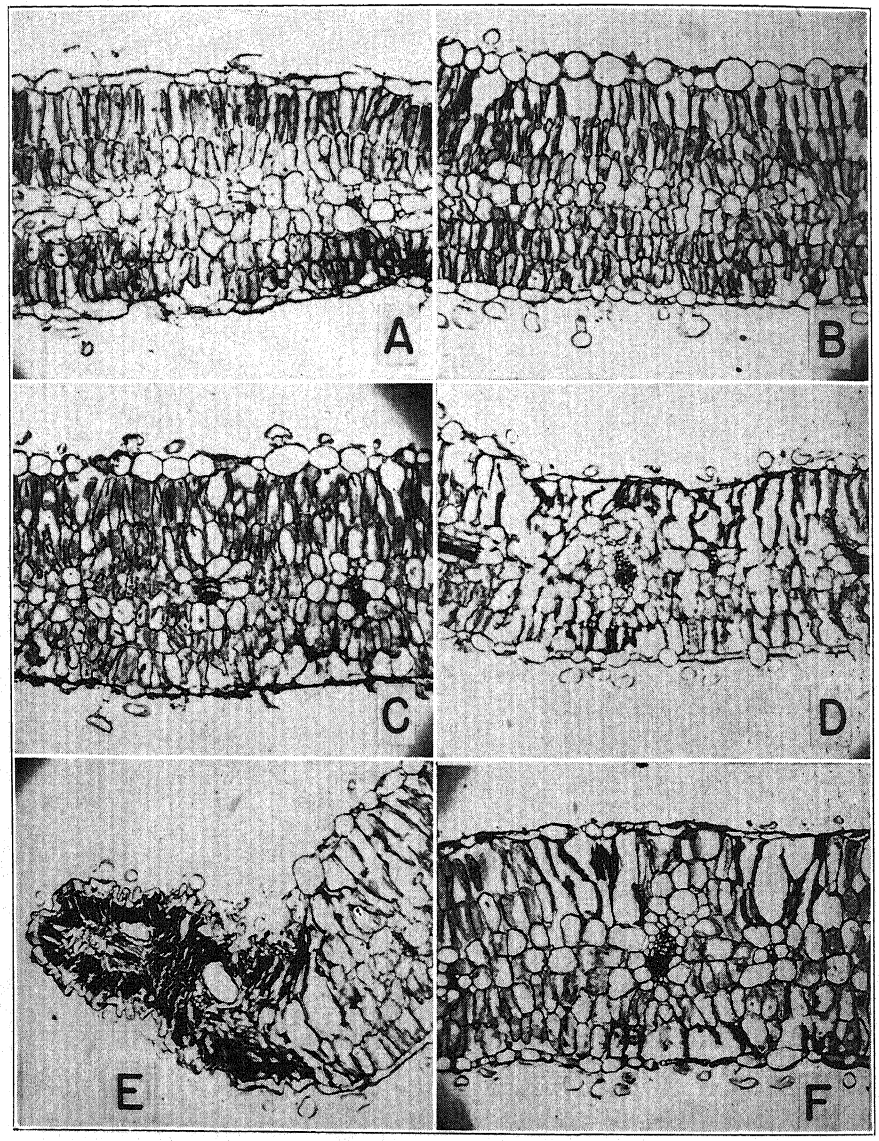


FIG. 2. Sections of oil-sprayed leaves. A. Uninjured leaf. B. 30 minutes after exposure to injurious amounts of oil spray. C. 3 hours after exposure. D, E. 24 hours after exposure. F. 48 hours after exposure. Magnification about 95×.

Material was obtained from two groups of plants in the Guayule Nurseries (Alisal), at Salinas, California. The first group was collected 14 days

after the seed was planted, and was still in the cotyledon stage. The second group had grown for about 8 weeks and had produced about a dozen leaves. Cotyledons and leaves from both groups were collected within 1 minute following exposure to an injurious spray (controls) and again in 30 minutes, 2, 24, and 48 hours after exposure. Observations were restricted to the cotyledons and foliage leaves as the primary effects of the spray were not marked in other organs.

The collected material was fixed in form-propiono-ethanol, dehydrated by means of tertiary butyl alcohol, and stained by safranine-fast green and safranine-hematoxylin. The methods employed followed the procedures outlined by Johansen in *Plant Microtechnique*.

Externally the injury appeared as brownish spots. Cotyledons, however, were often completely withered by the oil. In foliage leaves of older plants the spots varied from a few involving only a small fraction of the leaf area to large spots covering the entire leaf.

Photomicrographs of sections of leaves and cotyledons appear in figures 1 and 2. Figures 1, A, and 2, A, are the "controls," which show no injury. Figures 1, B–E, and 2, B–F, show examples of the progress of the injury in cotyledons and foliage leaves. Signs of injury appear within 30 minutes after exposure (Figs. 1, B, and 2, B). These show palisade cells in early stages of collapse. The leaf tissues most affected were those that apparently came in direct contact with oil. These were the epidermis (Figs. 1, E, and 2, C, D) and the palisade just within the stomata (Figs. 1, D, and 2, B, F). Curiously enough, the trichomes, which appear only on the foliage leaves, were seldom affected. The injury was characterized first by a collapse and shrinkage of the entire cell including the cell wall (Figs. 1, C, D, and 2, B) and later by a more or less complete cytolysis (Figs. 1, E, and 2, D, E, F). Injurious effects appeared to have reached their maximum in 24 hours after exposure (Figs. 2, D, E). No indications of the invasion or extension of injuries by micro-organisms were observed.

GUAYULE RESEARCH PROJECT,
SALINAS, CALIFORNIA.

A SIMPLE NUCLEAR STAIN AND STAINING TECHNIQUE FOR HELMINTHOSPORIA

G. K. Parris

(Accepted for publication February 24, 1944)

While studying the nuclei of the vegetative cells, young conidiophores, and germinating spores of members of the genus *Helminthosporium*,[1] considerable difficulty was experienced with retention of the fungal material on a coverslip or slide during the staining procedures preparatory to microscopic examination. At first the method of Dickinson[2] was tried, which in brief consists of smearing a thin film of egg albumen on a slide, and then flooding it with a suspension of fungal inoculum. The fungal material is allowed to settle for a minute, the slide drained, and put aside to dry. Dickinson says "This drying process was found to be the most critical part of the method. The slide must be allowed to dry in order to coagulate the egg albumen and so fix the inoculum to the slide, but it must not be allowed to dry sufficiently to kill the fungal material." Often, when this procedure was followed, and the slide with its fungal material placed in Flemming's solution and thence into Heidenhain's haematoxylin, the film of albumen would become unstuck, and the slide and all preparatory work connected with it rendered worthless. Furthermore, with Helminthosporia, spore production often would not take place on an egg albumen substrate.

Dickinson's method finally was abandoned and a search made for some other technique. This note gives the details of the method finally adopted; with certain modifications it should be adaptable to other fungi.

From experience it was learned that most Helminthosporia will produce spores on corn-meal agar, made either from commercial material containing dextrose, or according to Shear's formula, in which dextrose is not included. While a particularly recalcitrant isolate may refuse to sporulate on corn meal in a Petri dish, it usually can be induced to do so if the method to be described is followed. Petri dishes are lined at top and bottom with filter paper, which is then moistened with tap or distilled water. The dishes need not be sterilized, and sterilized water need not be used, for just as good results were obtained with well-washed dishes as with sterilized ones. In the bottom of each dish, resting on the wet filter paper, place 2 pieces of glass rod, each about $\frac{1}{2}$ to $\frac{3}{4}$ the width of the bottom. Using chemically clean slides, grasp a slide at one end with a flamed forceps, and dip the slide for $\frac{3}{4}$ its length into 95 per cent alcohol, and flame. Have handy a flask of melted corn meal agar. As soon as the alcohol on the slide has burned off, remove the cotton plug from the flask of agar, flame the mouth of the flask in the usual manner, and pour a small quantity of the agar on to the surface of the

[1] Research performed while on sabbatical leave from the University of Hawaii, Honolulu, T. H., at the University of Missouri, Columbia, Mo.

[2] Dickinson, Sidney. The nature of saltation in Fusarium and Helminthosporium. Minnesota Agr. Exp. Stat. Tech. Bul. 88: 1–42. 1932.

slide not grasped by the forceps, with the slide in a horizontal position. Tip the slide and allow the excess agar to run off into a container. If the slide is hot, more agar may be left on the slide than if the slide is moderately cool, for in cooling, the slide in its former condition will dry off a considerable amount of the residual agar. One should attempt to obtain a thin, even film of agar over the surface of the slide, which is next placed in the Petri dish so that it rests on the glass rods. The dish, as is now obvious, is merely a moist chamber, when covered. As soon as the agar has cooled, its surface is inoculated, usually in the middle.

By microscopic examination of the inoculated slide in 24 to 72 hours, it may be determined if spore germination has occurred, or if young conidiophores are present. The writer has found that an inoculated slide may be viewed under the microscope one or more times, each time requiring removal from its "moist chamber," without much danger of contamination from the air.

Staining to show the nuclei in Helminthosporia is easy and rapid when Maneval's[3] acid fuchsin stain is used.[4] For convenience, the ingredients of this stain are repeated:

Phenol, 5 per cent aqueous	30 cc.
Glacial acetic acid, 20 per cent aqueous	8–10 cc.
Ferric chloride, 30 per cent aqueous	4 cc.

To the above add 1–2 cc. of a 1 per cent aqueous solution of acid fuchsin.

To the slide on which the *Helminthosporium* sp. is sporulating or germinating, add enough acid fuchsin stain to flood the fungal material. This may be performed with impunity shortly after spore germination; to wash off the spores is extremely difficult. After 30 seconds to 3 minutes, depending on the material and the intensity of staining desired, drain off the excess stain and flood the slide with tap water. Wash one or more times with tap water, each time allowing the water to stand on the slide for 3 to 10 seconds; the number of washings is determined by the retention of the stain, which can be watched with the naked eye or with the microscope. Here, experience is better than any written instructions. Add one to two drops of lactophenol and cover with a cover slip. Examine under oil. The nuclei stain bright pink, the remainder of the cell and its contents, hardly at all; the agar does not retain much dye, and visibility should be excellent.

The lactophenol mentioned above is prepared in the usual manner, viz., distilled water 20 cc., phenol crystals (warmed until melted) 20 cc., lactic acid 20 cc., glycerin 40 cc. Preparations mounted in lactophenol and ringed with paraffin have been kept in useful condition for months, though the acid

[3] Maneval, W. E. Staining bacteria and yeasts with acid dyes. Stain Technology 16: 13–19. 1941.

[4] The writer wishes to express his appreciation to Dr. W. E. Maneval of the University of Missouri for suggesting the use of this stain and for helpful hints in staining technique.

fuchsin does decolorize slowly. This is sometimes a good point, for an overstained slide when kept for a month to 6 weeks may show later all that was originally desired.

VIRGINIA TRUCK EXPERIMENT STATION,
NORFOLK, VIRGINIA.

PHYTOPATHOLOGICAL NOTES

Tulip Blight Controlled by Organic Sulphurs.[1]—Tulip blight (*Botrytis tulipae* (Lib.) Lind) has long been prevalent and destructive in the Pacific Northwest. Commercial-bulb farmers have relied upon Bordeaux sprays for control, but often more damage has occurred from copper burning than from fungus attack.

In an attempt to obtain a more suitable fungicide, spraying experiments were initiated during the 1941–1942 bulb season with Bordeaux (2:4:50 and 4:4:50, the latter with and without Penetrol), copper phosphate, Coposil, Tennessee copper 26, Greenol, manganese-iron bordeaux, Lysol, potash-sulphur-resin, silver nitrate (2 formulas, silver-lauryl sulphate and silver-manganous sulphate[2]), Spergon, phenothiazine, malachite green (in

TABLE 1.—*Average results of 1943 spray trials for the control of Botrytis tulipae on four replications each of three tulip varieties*

Material	Concentration	Average number of lesions per replication[a]	Average degree of burning per replication[b]
Check (water)		115.9	0.0
Fermate	2 lb.: 100 gal.[c]	56.2	1.0
Thiosan	1½ lb.: 100 gal.[c]	93.7	0.7
Bordeaux	8: 8 : 100 gal.[c]	89.1	2.7
Copper oxalate	3 lb.: 100 gal.[c]	126.7	2.4
Silver nitrate	[d]	127.0	0.6
Sulphur dust	[e]	116.6	0.6

[a] Experiment statistically significant. The least difference required for significance is 57.4.

[b] Degree of burning rated from "0" (no burning) to "5" (severe burning).

[c] Penetrol added at rate of 1⅔ pt. per 100 gal. as a wetting and sticking agent.

[d] 1.195 g. silver nitrate, 3.79 g. manganese sulphate, 6.8 g. hydrated lime, 3.78 g. Vatsol OT per gal.

[e] 75 per cent sulphur, 24.9 per cent hard wheat flour, 0.1 per cent Vatsol K.

oil emulsion and in water), Thiosan and Fermate. These were applied in 4 replications each to the varieties Rev. H. Ewbank, William Pitt, and Rose Copeland. The number of Botrytis lesions were counted on 20 representative leaves in each replication at the end of the season. Fermate and Thiosan, which averaged 15.3 and 16.7 lesions per replication, respectively, were superior to all other materials. Bordeaux (4:4:50 without Penetrol) averaged 47.9 lesions, whereas the check averaged 101.4 lesions per replication.

Spray trials were repeated in the spring of 1943, using the most promising materials of the previous season as well as a sulphur dust and a copper oxalate spray. Three applications were made at 10-day intervals, beginning April 17, and again using 4 replications on the same three tulip varieties.

[1] Published as Scientific Paper No. 579, College of Agriculture and Agricultural Experiment Stations, State College of Washington, Pullman, Washington.

[2] Nielson, L. W., and C. E. Williamson. The composition and field performance of some silver sprays. Phytopath. 32: 1026–1030. 1942.

Results (Table 1) again demonstrate the superiority of Fermate over all other materials.

The greater degree of infection in these trials over those in 1942 is partly, if not entirely, attributable to the fact that the first spray application was delayed until the tulips had become generally infected. Fermate caused some burning when applied with a knapsack sprayer in the 1943 plot tests, but none when it was applied with a power sprayer to tulips in commercial fields by two farmers.

Fermate and Thiosan are closely related, both being derivatives of dithiocarbamic acid. The active ingredients in Fermate and Thiosan are ferric dimethyldithiocarbamate and tetramethyl thiuramdisulphide, respectively. The latter material was tried in 1938 in Holland (under the name Tulisan)[3] and found promising against tulip blight.

Fermate often produces a splotchy appearance on the plant when applied with a knapsack sprayer. Therefore, Thiosan, which is less noticeable, appears to be preferable for home flower gardens. Fermate, because of its superior control quality, will be recommended for trial in commercial fields in 1944. Four applications of a 2 lb.: 100 gal. solution at 10-day intervals, beginning as soon as the leaves are 3 to 4 inches high, should be adequate under most conditions.—C. J. GOULD, Western Washington Experiment Station, Puyallup, Washington.

Dodder on Flax and its Control.[1]—In 1941 the important hemiparasitic dodder (*Cuscuta*) was observed for the first time on flax in Peru (Mid-coast section: Cañete and Lima). Losses were sometimes serious because the dodder prevented development of the flax plants, made various cultivating processes difficult, and impeded proper retting. In some fields practically 100 per cent of the plants were attacked, the ramifications of the dodder interlacing with the flax plants to form a virtual mattress.

The species of *Cuscuta* which attacks flax in Peru is *C. indecora* Chois., according to the determination made by Dr. S. F. Blake, of the U. S. Department of Agriculture, Washington, D. C., to whom the writer expresses his gratitude.

A survey of all available foreign literature with respect to control reveals only preventive measures such as the use of clean seed. The problem in Peru, however, is not only a matter of future control but of finding a killing agent for the parasite. El Servicio de Fitopatologia of La Molina Experiment Station, of which the writer is head, undertook to find a substance capable of destroying plants of *Cuscuta* without injuring flax. The problem is very difficult, since plants of dodder are very resistant and those of flax very sensitive to various chemical agents. After testing a large number of different chemicals in varying concentrations, we found that very satis-

[3] Poeteren, van, N. Verslag over de werkzaamheden van den plantenziektenkundigen Dienst in het jaar 1938. Versl. Plziekt. Dienst Wageningen **93**: 1939. (Abs. in Rev. Appl. Myc. **19**: 194–195. 1940.)

[1] Translated from the Spanish by Laura M. Hamilton, St. Paul, Minnesota.

factory results could be obtained with an aqueous solution of sodium hydroxide (NaOH) at concentrations varying from 8 to 10 parts per 1000, depending on the stage of development of the flax.

Dosage: When young flax plants are treated, always use a concentration of 8:1000, applying 1 litre of solution to each 4 sq. m. or 25 hectolitres per hectare. When the flax is well developed, use a concentration of 10:1000, applying 1 litre of solution to each 3 sq. m. or approximately 33 hectolitres per hectare.

Since commercial soda (NaOH) is readily soluble in water, the quantity of soda to be used may first be dissolved in a small quantity of water and then to this solution may be added the amount of water necessary to bring it to the desired volume.

Application: A single application is sufficient if it is made in accordance with the instructions given here, which are based on results of a large number of trials. These instructions must be carefully followed to attain perfect control of *Cuscuta* and avoid burning of the flax plants.

1. It is of prime importance to apply the solution with a power sprayer which has a pressure of 300 lbs., taking special care that the pressure does not diminish during the spraying operation.
2. The stream produced by the power sprayer on the flax plants should be inclined from above, downward, and at an angle of 45° with the vertical.
3. The spray should be as fine as possible.
4. Special care should be taken to apply the spray as uniformly as possible.
5. The solution should be applied very lightly on flax plants.
6. Spraying should be done when there are no air currents.
7. After being sprayed once, plants should not be sprayed again, since a repetition of the application will burn the flax plants.
8. The concentration of the solution should not exceed 10:1000 as a maximum.
9. To avoid burning the flax, great care should be taken when the liquid in the spray machine is nearly gone, since the concentration of the solution is likely to be higher.

By following these instructions faithfully, it is easy to obtain up to 90 per cent control of *Cuscuta.* It should be kept in mind, however, that the method of application is of greater importance than the amount of material used.—G. GARCIA RADA, Estacion Experimental Agricola de La Molina, Lima, Peru.

ANNOUNCEMENT

The American Phytopathological Society will not meet with the American Association for the Advancement of Science in September. The Society will hold its 36th Annual Meeting and War Conference December 9–11, 1944, at the Netherland-Plaza Hotel, Cincinnati, Ohio. Abstracts of papers to be presented at the meetings must be in the hands of the Secretary, Dr. C. C. Allison, by October 15.

NOTICE

NATIONAL ROSTER OF SCIENTIFIC AND SPECIALIZED PERSONNEL

In the light of recent Selective Service directives which will result in the induction of many thousands of professionally and scientifically qualified young men under the age of 26, it is important that these particular individuals who will enter the armed forces immediately notify the Roster of the branch of the armed forces they are entering, the date and place of their induction and, after induction, their serial number. Obviously the Roster, as a civilian agency of Government, has no responsibility in the assignment of its registrants within the armed forces. At the request of the War and Navy Departments, however, the Roster does furnish advice to the Office of the Adjutant General, War Department, and the Bureau of Naval Personnel, Navy Department, concerning the specialized training and qualifications of those of its registrants who are inducted into the Army or Navy. This information is used as an aid in determining the initial military assignment of the partcular individual.

It is conceivable that it may become imperative in the future—near or distant—to withdraw a number of professionally and scientifically qualified men from the armed forces in order to assign them to important research or production work in civilian war industry. The Roster's records are sufficiently detailed to permit intelligent selection of persons possessing professional qualifications in practically every kind of specialized field and it would probably be called upon to assist in any such assignment. It is important, therefore, that persons possessing professional or scientific qualifications register with the Roster and advise it immediately concerning any change in their status. Communications should be addressed to the National Roster of Scientific and Specialized Personnel, 1006 U Street, N.W., Washington 25, D. C.

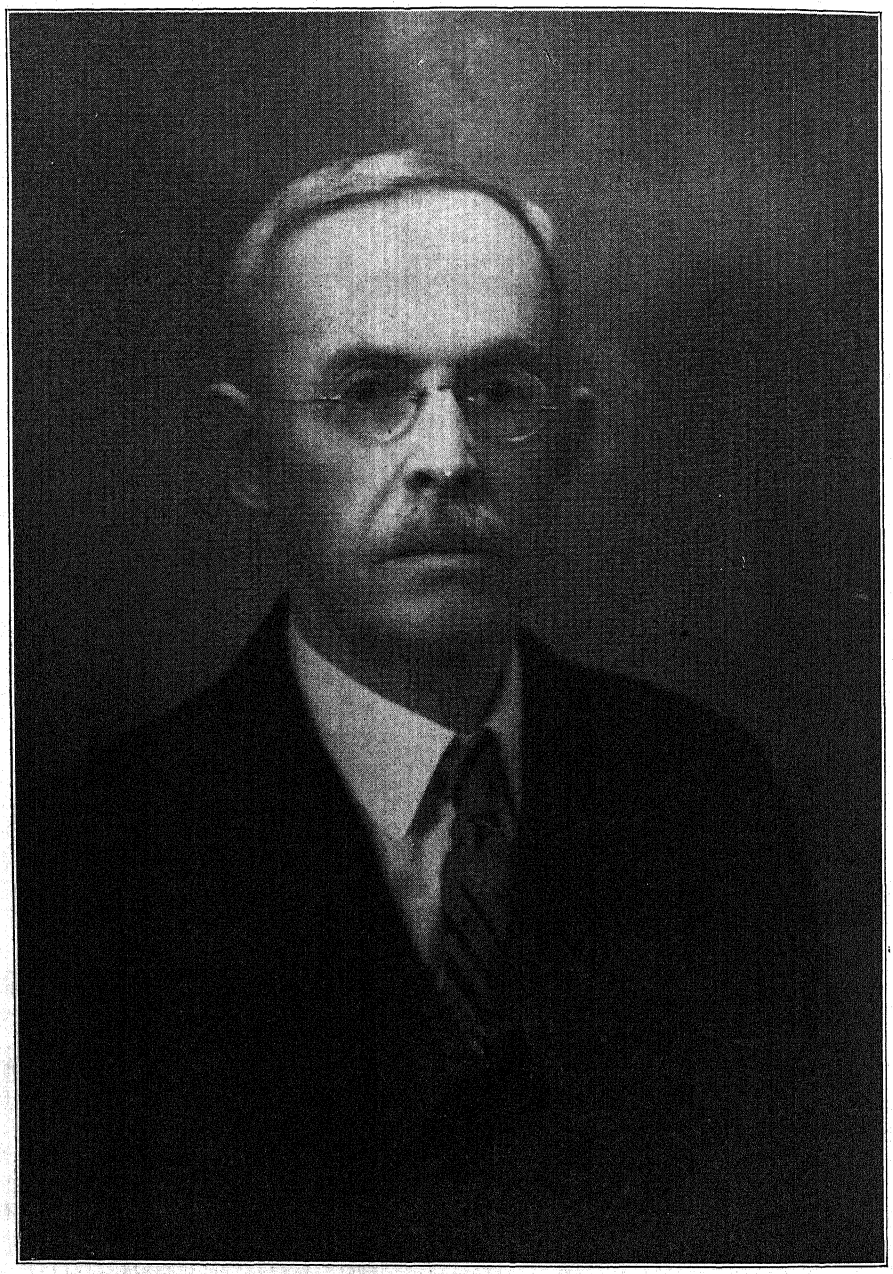
WILLIAM POLLOCK FRASER
1867–1943

J. H. CRAIGIE

William Pollock Fraser, Professor-Emeritus of Biology in the University of Saskatchewan, died at his home in Saskatoon on the morning of November 23, 1943. For several years, he had been afflicted with a heart ailment, which curtailed considerably his activities, but, with the devoted assistance of his wife, he continued his scientific work, at times from a sick bed, until a few days prior to his death. His passing is a pronounced loss to botanical science and to a wide circle of associates, students, and friends who held him in esteem and affection.

Dr. Fraser was born in 1867 at French River, Pictou County, Nova Scotia. He was the son of Alexander and Anna (Pollock) Fraser, and had an older sister and two younger brothers, both of whom died in childhood. He attended the local school—an actual "little red school house." Through the accidental death of his father, he was left in early life to take charge of the home farm. At the age of twenty-one, he yielded to his desire for further education, and, with his mother (his sister having now married), he left the farm and entered New Glasgow High School. He later attended Pictou Academy—from which he graduated in 1896 with senior matriculation—and the Normal School at Truro, N. S. As he had to pay his own way, these years of study were interspersed with years of teaching. In 1899, he enrolled in Dalhousie University, where he studied for two years. He was Principal of Westville High School from 1901 to 1903 and, in the latter year, joined the Staff of Pictou Academy as Instructor in Natural Science. In 1905, he resigned this position to attend Cornell University, from which he received the A.B. degree in 1906. He continued his studies there, and, in 1907, was re-appointed to his former position in Pictou Academy. From Dalhousie University, he received the B.A. degree *ad eundem* in 1907, and the M.A. degree in 1910. In January, 1912, he was appointed to the Staff of Macdonald College as Lecturer in Biology and, in this year, he married Miss Alice Adele McRae. Two years later, he was promoted to Assistant Professor of Biology.

After the disastrous outbreak of stem rust in Western Canada in 1916, the Dominion Department of Agriculture requested him to study the cereal rust situation in that area during the summer of 1917 and of 1918, and, in 1919, appointed him as Officer-in-Charge of the newly established Dominion Laboratory of Plant Pathology at the University of Saskatchewan, Saskatoon, Sask. In the same year, the University of Saskatchewan granted him the M.A. degree *ad eundem* and appointed him, on a part-time basis, as Lecturer in Biology. These appointments he held concurrently until 1925, when he severed his connection with the Department and accepted the position of Professor of Biology in the University. On his retirement in 1937,



WILLIAM POLLOCK FRASER
1867–1943

the University, in recognition of his services, honored him with the LL.D. degree and appointed him Professor-Emeritus of Biology. He was a member of the Associate Committee on Field Crop Diseases of the National Research Council and the Dominion Department of Agriculture. He was also a member of the American Phytopathological Society, a Fellow of the American Association for the Advancement of Science, and a charter member of the Mycological Society of America, of the Canadian Society of Technical Agriculturists, and of the Canadian Phytopathological Society. In the latter Society, he served as Vice-President from 1929 to 1931, as President from 1931 to 1933, and was elected in 1940 to honorary membership.

Dr. Fraser will be remembered for his utter genuineness of character, the keystone of which was his absolute loyalty to truth. He was wholly incapable of subterfuge or dissimulation. Sincere and reserved, he sought out the quiet lanes of life. He craved neither popularity nor distinction. His manner of life was simple, his self-effacement, complete, but his nobility of character and disciplined mind compelled deference and inspired confidence. It was well said of him that he "wore the ornament of a quiet and humble spirit." He was a teacher of wide experience—in elementary, high school, and university work; and just as in his social relations he influenced by example rather than precept, so in his teaching he placed the emphasis more on demonstration than on formal lectures. He was a master of laboratory instruction and was noted for the excellence of his demonstrational material. In research, he worked with his students and associates, as collaborator rather than director, and gave them generously of his time and ability. In return, their regard for him partook of idealization, and they accorded him a respect bordering on reverence.

A born naturalist, Dr. Fraser was familiar with birds and insects, but chose plants as his special field of study. He was an indefatigable collector, and spent most of his spare time in collecting and classifying plants, cryptogams as well as phanerogams, with the object of building up herbaria in the institutions with which he was connected. Although for some years his work was mainly concerned with cereal disease investigations, he retained his interest in the native flora and subsequently devoted an increasing proportion of his time to a study of it. On his retirement in 1937, he undertook the re-organization of the phanerogamic herbarium of the university and also a revision of the list of Saskatchewan plants earlier published by him and Dr. R. C. Russell, neither of which tasks he was able quite to complete.

His direct contribution to plant pathology has been definite and substantial, although at no time was his work entirely restricted to it. He was the pioneer in plant rust research in Canada, his cultural studies of the heteroecious rusts being outstanding. He recognized early the importance of physiological specialization in the cereal rusts and its implications in respect to plant breeding for rust resistance. Likewise, he recognized, and stressed, the urgency of undertaking plant breeding for the production of

rust resistant varieties of cereals. While in charge of rust investigations in Western Canada, he initiated and conducted an annual survey of the physiologic races of stem rust present in that area, and also epidemiological studies on the sources and spread of cereal rusts. By the time he relinquished the active direction of these and related investigations, the general plan of subsequent rust research in Canada was fairly well defined. Other contributions might be mentioned, but probably his greatest contribution to plant pathology, as well as to other fields of botanical science, was indirect, that is to say, through the example of his complete and disinterested devotion to scientific truth and the inspiration he imparted to his students and associates in research.

He was author or co-author of the following publications:

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A THECAPHORA SMUT ON POTATOES

M. F. Barrus

(Accepted for publication March 25, 1944)

Barrus and Muller[1] described a disease of potato tubers, known locally as *buba* disease, occurring in the Andes Mountains of Venezuela on Andean varieties (*Solanum andigenum* Juz. et Buk.). It is characterized by the presence of warts or lumps on the surface and by numerous brown specks throughout the flesh. These specks prove to be cavities filled with rusty

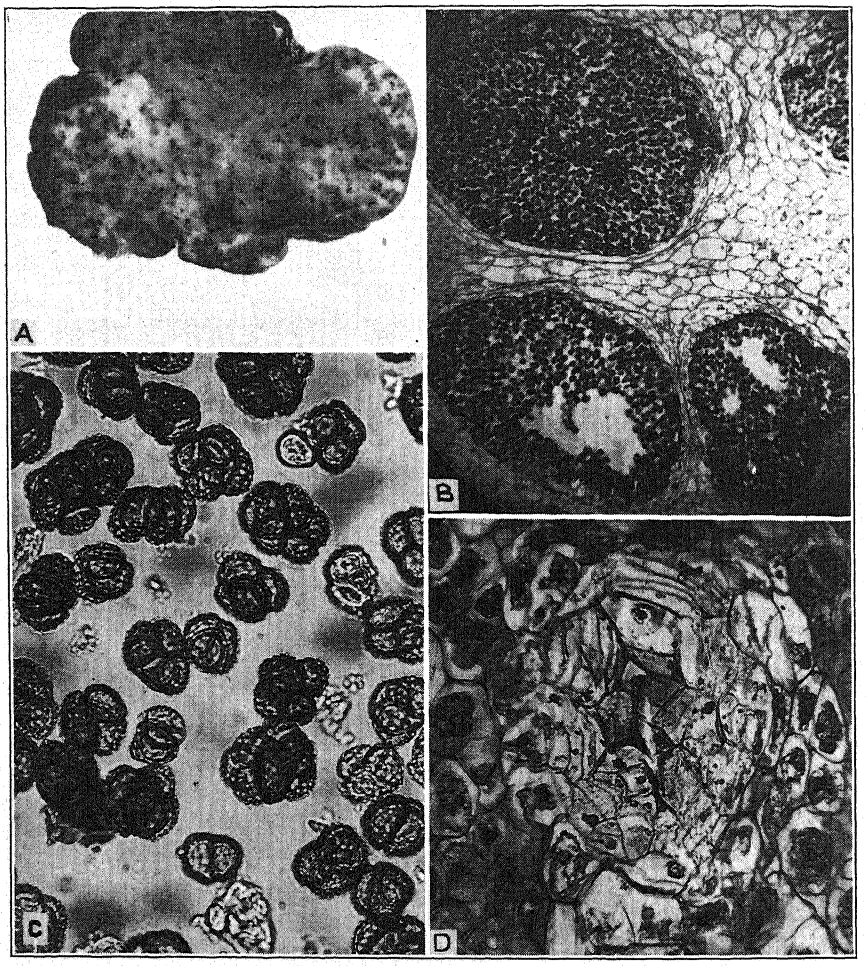


Fig. 1. *Thecaphora solani* on *Solanum andigenum*. A. Longisection of a tuber showing lobed surface and internal specking, ×1.2. B. Sori of smut in flesh, ×31.7. C. Sporeballs, ×328.5. D. Young sorus showing phellem, ×166.25. Photographed by W. R. Fisher. B and D from stained slides made by Ruby Rice.

[1] Barrus, M. F., and A. S. Muller. An Andean disease of potatoes. Phytopath. 33: 1086–1089. 1943.

brown sporeballs consisting of 1 to 8 spores prominently and abundantly verrucose on the exposed surfaces (Fig. 1, A, B, and C).

Since that article was prepared, I have had an opportunity to examine other material sent by J. Camero Zamora from the same locality as well as material sent by John A. Stevenson of the United States Department of Agriculture from Peru, from Ecuador, and from the original collection by Chardon in Venezuela. Attempts have been made to germinate the spores, but thus far without success. It was first thought impossible to determine the pathogen without observing the type of spore germination but, after

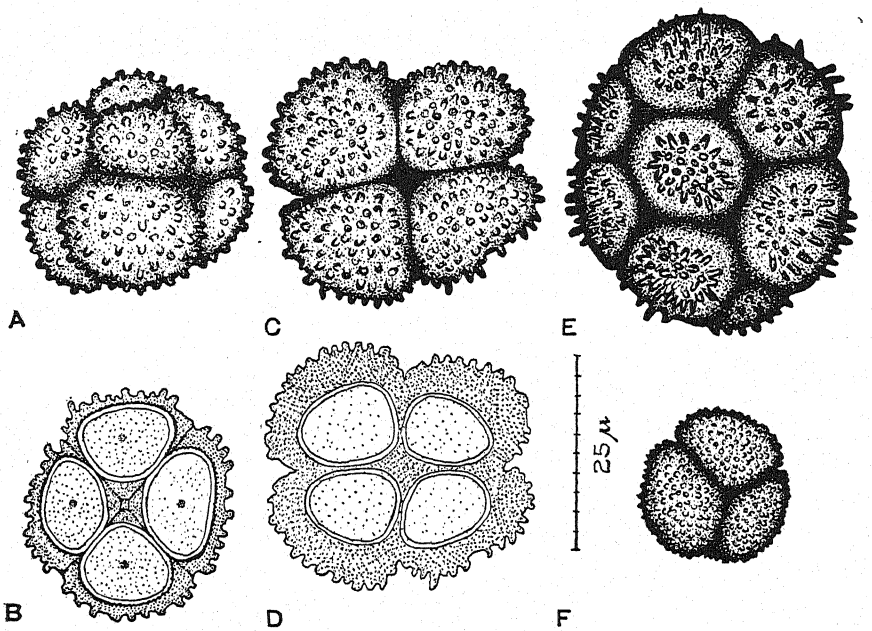


FIG. 2. Sporeballs of four species of *Thecaphora* showing similarity in shape, size, and character of outer wall. Drawn to the same scale with aid of camera lucida by Carlos Garces. Dry spores mounted in Shear's mounting fluid, except B which is from a stained tissue section made by Ruby Rice. A. *T. solani* Bar., CUPP 33247; B. *T. solani*, CUPP 29427; C. *T. pustulata* Clin. CUPP, Fungi of Puerto Rico 458; D. *T. pustulata*, optical section of C; E. *T. deformans* D. et M., Zillig Ust. Eur. 84; F. *T. trailii* Cke. Krieg. Fung. Sax. 1451.

making a comparison of the sporeballs of this fungus with those of other species of smut fungi, I have come to the conclusion that it is a new species of *Thecaphora*. This conclusion is based on the similarity to several other species of this genus in size, shape, and color of the sporeballs, the number of spores composing them, and the thick verrucose wall on their exposed surfaces (Fig. 2). The spores of these other species have also been germinated with difficulty or not at all.

The sporeballs of this new species of *Thecaphora* in the several collections are very similar but the average measurements differ slightly. The spores have a definite inner wall up to 1 μ thick. The spore measurements given

here do not include the outer wall because of the difficulty of determining its exact limits. This varies in thickness from about 0.5 μ or less on the side adjacent to other spores in the ball to as much as 1.5 to 4 μ on the exposed surfaces.

As Barrus and Muller described the disease and the fructifications of the pathogen rather completely, there is given here only a description of the fungus sufficient to establish its position. In naming it, consideration is given to the possibility of its being classified elsewhere when more information regarding its development is available.

Thecaphora solani n. sp.

Sori numerous, embedded in the cortex and pith of warty, lobed, or otherwise misshapen tubers; subcircular, oval or irregular in cross section; up to 1 mm. long; containing rusty to dark-brown spore masses; occasionally two or three sori adjoining each other; surrounded when young by phellem (Fig. 1, D): sporeballs yellowish to rusty brown, dusty after exposure to air, mostly subspheric to ovoid, 12–48 × 12–35 μ, consisting of 2 to 8 adherent spores (usually 3 to 6 and occasionally a single one); spores subspheric to ovoid or somewhat angular in optical section, 7.5–20 × 8–17.5 μ, with contiguous walls smooth and the free wall rounded, thick, and abundantly verrucose.

Habitat: In tubers of an Andean variety of *Solanum andigenum* Juz. et Buk.

Type: CUPP[2] 29427, collected by Barrus and Muller at Mucuchíes, State of Mérida, Venezuela, on November 19, 1939. Type material has also been distributed to the following herbaria: Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland; New York Botanical Garden, New York City; Farlow Herbarium, Cambridge, Massachusetts; Pennsylvania State College of Agriculture, State College, Pennsylvania; University of Michigan, Ann Arbor, Michigan; University of Toronto, Toronto, Canada; University of Wisconsin, Madison, Wisconsin; Missouri Botanical Garden, St. Louis, Missouri; Royal Botanic Gardens, Kew, England; and British Museum of Natural History, London, England.

Distribution: Andes Mountains at elevations of 2500 to 3300 meters in Venezuela, Ecuador, and Peru.

Other Material Examined: BPI[2] 71386 and CUPP 33244, collected by Carlos E. Chardon near Mérida, Venezuela, in August, 1932, and sent by him to the United States Department of Agriculture, Bureau of Plant Quarantine, Washington, D. C.; BPI 71388 and CUPP 33245, collected by J. Soukup, Puno, Peru, in April, 1940; BPI 71387 and CUPP 33246, collected by Luis Rodriguez Lz. at Alausí, Ecuador, in January, 1943; and CUPP 33247, collected by Carlos Gonzalo Salas at Mucuchíes, Venezuela, on April 28, 1934, at the request of J. Camero Zamora of the Agricultural Experiment Station, El Valle, Venezuela.

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ITHACA, NEW YORK.

[2] CUPP refers to the herbarium of Cornell University, Department of Plant Pathology, Ithaca, N. Y., and BPI to the Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

THE OCCURRENCE OF A VARIANT IN RHIZOCTONIA SOLANI[1]

L. H. PERSON

(Accepted for publication February 17, 1944)

In the course of studies of various isolates of *Rhizoctonia solani* Kühn, a sector was found in a culture, isolated originally from a lesion on a bean stem (Fig. 1). Transfers from the sector, when compared with the original culture, were found to be distinct, the mycelium being somewhat lighter in color and with more aerial growth. As reports of variants in this fungus seem relatively rare, being confined to ones by Ullstrup[2] and LeClerg,[3] a

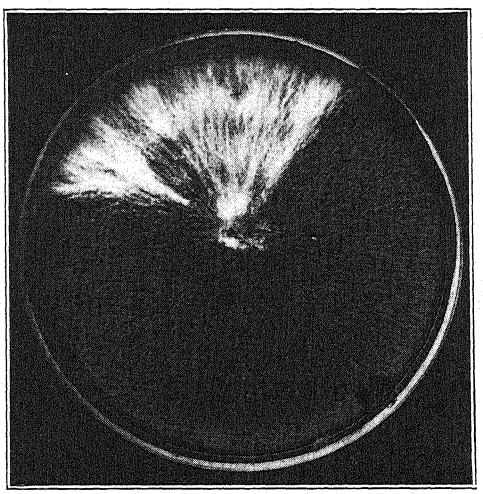


FIG. 1. Parent culture of *Rhizoctonia solani* and sector variant.

comparative study was made of the temperature relations and pathogenicity of the parent culture and variant.

It is realized that the mode of origin of the sector may be questioned, as the original culture was a tissue isolate and may not have come from a single hypha. This, however, seems improbable, as the sector did not appear until after the original culture had been studied for 3 years under a variety of conditions.

TEMPERATURE RELATIONS

The radial growth of the original culture and variant were compared on bean-pod agar at temperatures between 15° and 34° C. The results shown in table 1 indicate that the parent culture grew more rapidly than the variant at 20° and 24°, about the same at 29°, and somewhat more slowly at 34°.

[1] Excerpt from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted by the University of Minnesota, August, 1937.

[2] Ullstrup, Arnold J. Leaf blight of China Aster caused by *Rhizoctonia solani*. Phytopath. 26: 981–990. 1936.

[3] LeClerg, E. L. Studies on a cultural variant of *Rhizoctonia solani*. Phytopath. 29: 267–274. 1939.

TABLE 1.—*Growth of Rhizoctonia culture and its variant as shown by diameter of colony in mm. after 50 hours (average of 2 tests)*

Culture	15° C.	20° C.	24° C.	29° C.	34° C.
Original culture	25	59	88	83	65
Variant	24	47	76	83	69

PATHOGENICITY

Inoculations were made in sterilized soil in the greenhouse to compare the pathogenicity of the parent culture and variant. The method was the same as given elsewhere.[4] To determine the pathogenicity, the stand in per cent and the severity of stem lesions were used. To measure the severity of the stem lesions caused by a culture, each plant was placed in 1 of 5 classes ranging from 0 to 4, zero indicating no stem lesions and 4 indicating that the plants were killed. The degree of infection was then calculated as the average of the value of the stem lesions on all the plants inoculated with a culture. The results of 3 tests (Table 2) showed that the variant was less pathogenic than the parent culture.

TABLE 2.—*Comparative pathogenicity of original culture and variant to beans (average of 3 tests of 100 seeds each)*

Inoculum	Planted in inoculated soil		Soil inoculated after emergence of plants
	Per cent stand	Degree of infection	Degree of infection
Original culture	52	2.21	3.13
Variant	69	1.01	1.69
Control	84	0.00	0.00

Since a difference between the original culture and variant occurred in pathogenicity on beans, 2 more tests were made, which also included soybeans, cowpeas, and English peas. The results (Table 3) indicate the variant was less pathogenic on each host than the original culture.

TABLE 3.—*Comparative pathogenicity of original culture and variant on beans, soybeans, cowpeas, and English peas (average of 2 tests of 100 seeds each)*

Inoculum	Stand in per cent			
	Beans	Soybeans	Cowpeas	English peas
Original culture	63	1	40	0
Variant	78	36	76	81
Control	92	56	94	94

[4] Person, L. H. Parasitism of *Rhizoctonia solani* on beans. In manuscript form.

SUMMARY

A sector variant, which occurred in an isolate of *Rhizoctonia solani* from snapbean, was compared with the original culture in growth rate at different temperatures and in pathogenicity. The variant differed in growth rate at certain temperatures and was less pathogenic than the original culture on beans, soybeans, cowpeas, and English peas.

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STUDIES ON LILY VIRUS DISEASES: THE MOTTLE GROUP

PHILIP BRIERLEY[1] AND FLOYD F. SMITH[2]

(Accepted for publication March 25, 1944)

Lily mosaic as reported by Guterman (6, 7) was chiefly a mottling disease, but the necrotic fleck symptoms in Easter lily (*Lilium longiflorum* Thunb.) were included without recognition of their separate etiology. McWhorter's reports (12, 14) of tulip breaking induced by mechanical inoculation with sap of various lilies encouraged the use of tulips (*Tulipa*



FIG. 1. Virulent-mottle (VCM) symptoms in Easter lily seedling. Note narrowed leaves and leafless interval in stem with normal leaves above and below. Inoculated Feb. 17, 1943. Photographed in 5-inch pot July 14, 1943, by Mead.

gesneriana L.) as test plants for lily viruses both at the Oregon Experiment Station and at the Plant Industry Station, Beltsville, Maryland. As shown later (3) *Lilium formosanum* Stapf is also a sensitive test plant for most lily viruses of the mottle type. Studies on the mottle viruses as well as on the necrotic-fleck complex (5) indicate that the mottle viruses are of major importance to some garden lilies, but of lesser importance in commercial

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[2] Entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.

Easter lily culture. This paper reports data on properties, host range, and vector relations of 3 lily-mottle viruses assigned to the tulip virus group, with comparative data on McWhorter's (15) Tulip viruses 1 and 2.

Data on physical properties, host range, and vector relations of the strong mottle virus of Easter lily (3) (CM or coarse mottle in our notation) have been accumulated since 1937. In 1939 McWhorter supplied samples of Tulip virus 1 and Tulip virus 2 (15) in tulips, and of a latent virus of lily (14) in *Lilium tigrinum* Ker-Gawl. A more virulent virus (VCM or virulent coarse mottle) appeared in 1942 in Creole Easter lilies (Fig. 1)

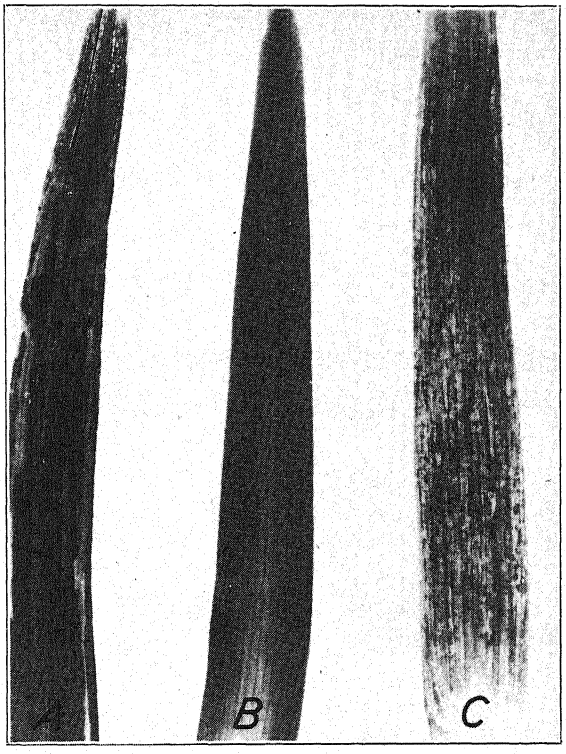


FIG. 2. Symptoms in *Ornithogalum thyrsoides* of (A) lily-virulent-mottle virus (VCM), and (C) Ornithogalum-mosaic virus, in comparison with (B) healthy control leaf. (A) and (C) transferred by *Myzus persicae*. ×½.

previously affected with the strong (coarse) mottle (CM). These 5 virus cultures were then compared in selected test plants, and parallel determinations of properties were made in *L. formosanum* to decide whether they must be considered distinct or if they may be interpreted as strains of one virus.

SOURCE OF VIRUS CULTURES

CM (coarse mottle), the common mottling virus of Louisiana Creole Easter lilies, isolated from one of these in 1937, passed through tulip, and repeatedly subcultured in *Lilium longiflorum* and *L. formosanum* seedlings.

VCM (virulent coarse mottle), transferred from a naturally affected Creole Easter lily to *Ornithogalum thyrsoides* Jacq. (Fig. 2) and thence to Easter lily seedlings by *Myzus persicae* Sulz., then subcultured mechanically in Easter lily and *Lilium formosanum* seedlings.

LT, a latent virus isolated from *Lilium tigrinum* furnished by McWhorter in 1939, and subcultured in *L. tigrinum* and *L. formosanum*. Inasmuch as this LT culture produces predominantly dark breaks in tulips, it should not be construed as typical of the latent virus of lily reported by McWhorter (14).

TABLE 1.—*Symptoms of 5 mottle viruses in 4 selected test plants*

Virus	Easter lily	*Lilium formosanum*	*Lilium tigrinum*	Clara Butt tulip
VCM	Coarse mottling, striping, twisting, and narrowing of leaves; occasionally flower distortion.	Yellowing, dwarfing, fine green islands on yellowish green ground.	Gray surface etch followed by general yellowing and death.	Mild leaf mottling; mixed type flower break, bleaching predominant.
CM	Coarse mottling only.	"	"	Strong yellow leaf mottling; mixed type flower break, bleaching predominant.
LT	Symptomless.	No yellowing; no dwarfing; upright habit; green island mottling.	Fine green mottling, soon masked.	Barely perceptible leaf mottling; mixed type flower break, intensification predominant.
TV1	Symptomless.	No yellowing; no dwarfing; upright habit; sparse and fine green islands.	Green mottling, soon masked.	Mild leaf mottling; mixed type flower break, bleaching predominant.
TV2	Symptomless.	No yellowing; no dwarfing; upright habit; coarse dark and light green mottling.	Gray surface etch only.	Mild leaf mottling; mixed type flower break, intensification predominant.

TV1, tulip virus 1 (15) isolated from Clara Butt tulip furnished by McWhorter in 1939, and subcultured in tulip and *Lilium formosanum*.

TV2, tulip virus 2 (15) isolated from Farncombe Sanders tulip furnished by McWhorter in 1939, and subcultured in tulip and *Lilium formosanum*.

GENERAL CHARACTERIZATION OF THE FIVE MOTTLE VIRUSES STUDIED

The principal test reactions by which these 5 viruses are distinguished are shown in table 1. The Easter lily mottle virus (CM) is characterized by coarse mottling without leaf distortion in this species (Fig. 3), by bleaching or color removing breaks in tulips (Fig. 4), and by its killing effect in tiger lilies (Fig. 5, A). This virus is apparently universal in Creole Easter

lilies. Some lines of this variety are typically symptomless, *e.g.*, the Norwood clon from Louisiana and a stock indistinguishable from Creole received from a Florida source. These symptomless stocks give the characteristic test reactions for CM in *Lilium formosanum* (Figs. 5, B, and 6, B), *L. tigrinum* (Figs. 5, A, and 7, A), and tulip (Fig. 4).

Virulent mottle virus (VCM) produces effects similar to CM in all test plants but two. This virus alone of the mottle group has been transmitted to *Ornithogalum thyrsoides,* inducing a conspicuous coarse green mottling (Fig. 2), distinct from the fine green mottling of Ornithogalum mosaic (18). In Easter lily, VCM produces yellow stripes as well as mottling (Figs. 1, 3), and also various leaf deformations. Some leaves are curved laterally, others

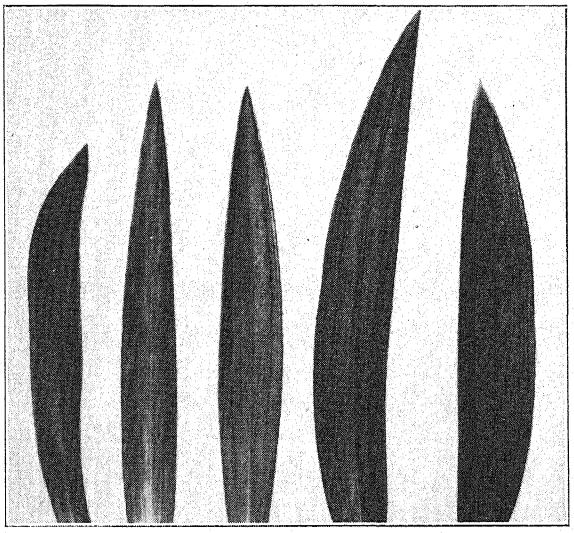


FIG. 3. Leaf symptoms in Easter lily. Left to right VCM, CM, LT, TV1, TV2. The last 3 symptomless, proved by indexing. Photographed by Mead.

are greatly reduced in width, and some are filiform. Occasionally Easter lily stems are bare of leaves for several inches with mottled leaves of nearly normal form above and below. When a zone of strong symptom expression occurs at bud stage, the flowers are variously deformed, with curled and narrowed perianth segments. VCM was first recognized in 1942, in several commercial fields in Louisiana, and in several greenhouses at Beltsville, Maryland, always in Easter lily stocks of previous CM history. Circumstances suggested a variant of CM rather than a new virus contaminant, and data subsequently obtained support this assumption. Survival of VCM from season to season in Creole lilies grown under glass has been low, 1 in 17 in one lot, the remainder reverting to CM symptoms.

A latent virus (LT) in tiger lily, obtained from McWhorter (14), produces dark intensifying flower breaks in tulip (Fig. 4), thus differing sharply from TV1 with which it agrees rather closely in other test-plant

reactions (Figs. 3, 5, A, B, 6, 7, 8). In McWhorter's original account (14), latent viruses transferred from Easter lily, *Lilium candidum* L., and *L.*

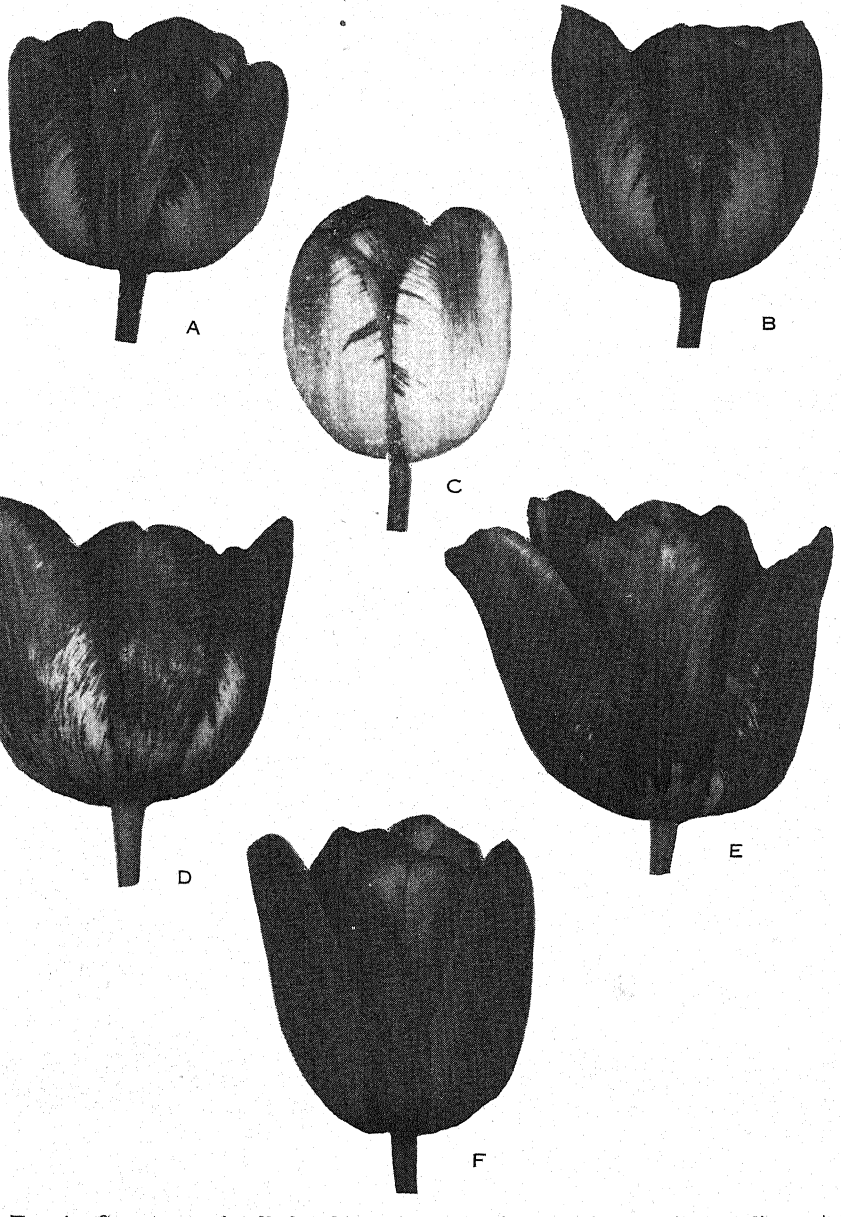


FIG. 4. Symptoms of tulip-breaking viruses in flowers of Clara Butt tulips. A, VCM; B, CM; C, TV1; D, LT; E, TV2; F, normal flower. A, D, E transferred by *Myzus persicae;* B, C transferred mechanically. Photographed February, 1943, current season symptoms.

tigrinum were considered identical with TV1, but he has later (16) pointed out cytological symptoms that distinguish some of them. The bleaching-

type breaks in tulips reported characteristic of "lily latent virus" in his first report (14), and pointing to identity with TV1, often result from inoculations from Easter lily and *L. candidum* into tulips, but not from this

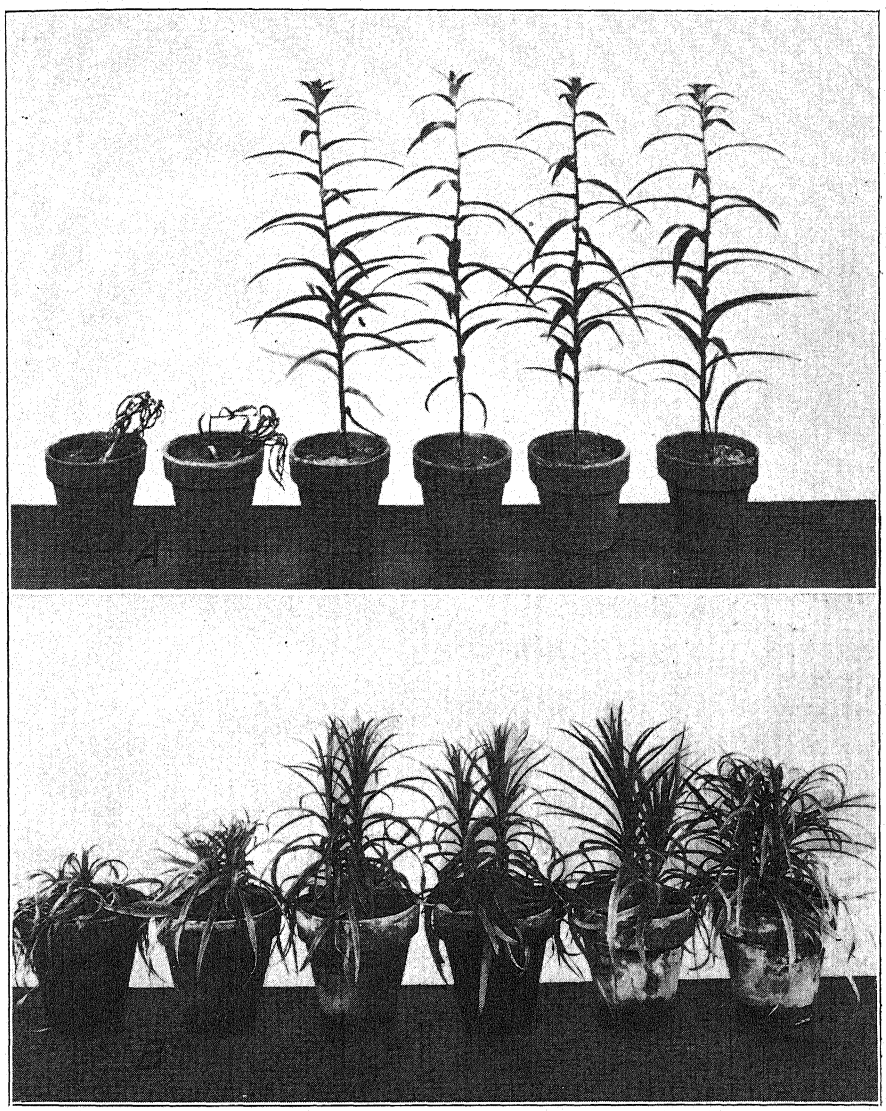


FIG. 5. Reactions of tulip-breaking viruses in (A) *Lilium tigrinum* in 4-inch pots inoculated Mar. 24, 1943, and (B) *L. formosanum* in 5-inch pots inoculated Mar. 25, 1943. Left to right VCM, CM, LT, TV1, TV2, control. Note VCM and CM are fatal to *L. tigrinum*, cause dwarfing in *L. formosanum*. Photographed May 8, 1943, by Mead.

L. tigrinum source. The virus we term LT in this paper, therefore, may be regarded as a sample of latent virus from *L. tigrinum* but not as type material of McWhorter's latent virus of lily (14). LT or a similar virus

is common but not universal in tiger lilies, stocks free from it having come to our hands from Maryland, New York, Oregon, and Vermont. Affected tiger lilies usually show a well-defined, mild, green leaf mottling in early stages of development, but become symptomless before flowering.

Tulip viruses 1 and 2 were segregated by McWhorter (15) as bleaching and intensifying components, respectively, which in mixture produce the average break or Rembrandt type in various Darwin tulips. These viruses, although uncommon in lilies, are obviously more or less closely allied to the lily-mottle group. Tulip virus 1 (TV1) differs from Tulip virus 2 (TV2) and from LT in producing bleaching breaks in tulip (Fig. 4) and differs from CM and VCM in failing to produce symptoms in Easter lily

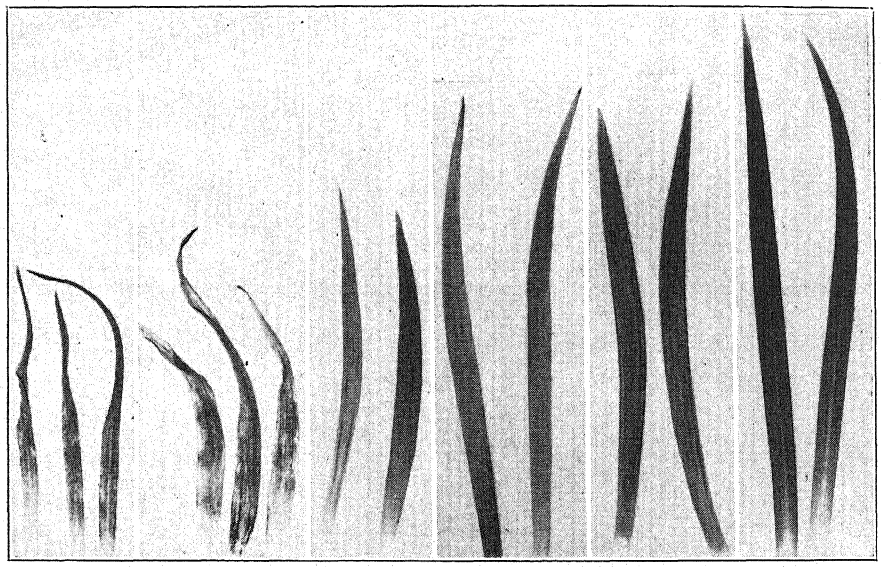


FIG. 6. Symptom expression of mottle viruses in leaves of *Lilium formosanum:* A, VCM; B, CM; C, LT; D, TV1; E, TV2; F, control. Photographed 36 days after inoculation.

(Fig. 3). TV2, a color-adding or intensifying type in tulip (Fig. 4), differs from the other viruses, except LT, here discussed in this respect. It is distinct from LT in the detailed symptoms expressed in both *L. tigrinum* (Fig. 7) and *L. formosanum* (Fig. 6).

The symptoms expressed in *Lilium formosanum* (Figs. 5, B, 6) fail to distinguish VCM from CM, but are recognizably different for the other 3 viruses studied. Inasmuch as VCM is known only from Easter lily, in which it is distinctive, *L. formosanum* is a valuable test plant for preliminary classification of unknowns. The CM and LT types encountered in lilies yield distinctive reactions in all the test plants listed (Figs. 3, 4, 5, A, B, 6, 7, 8). The sharpest distinction between these 2 virus types is found in *L. tigrinum* in which LT is symptomless after mild mottling in early growth, but which CM kills outright in 6 to 8 weeks (Figs. 9, 10, 5, A).

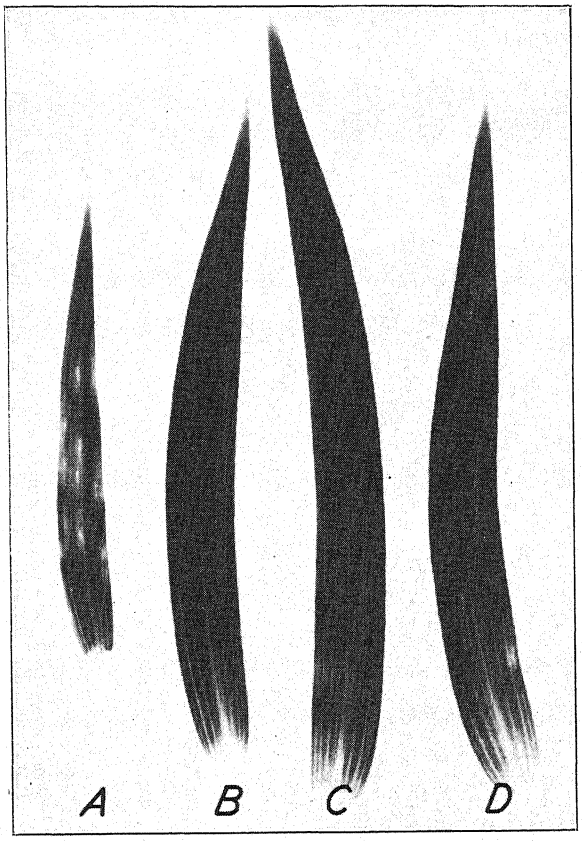


FIG. 7. Symptom expression of mottle viruses in leaves of *Lilium tigrinum:* A, lily-mottle virus CM (similar reaction from VCM not shown); B, LT; C, TV1; D, TV2.

Some virus cultures obtained in earlier indexing (3) were retained for a time and tested on Easter lily and tulip as well as *Lilium formosanum,*

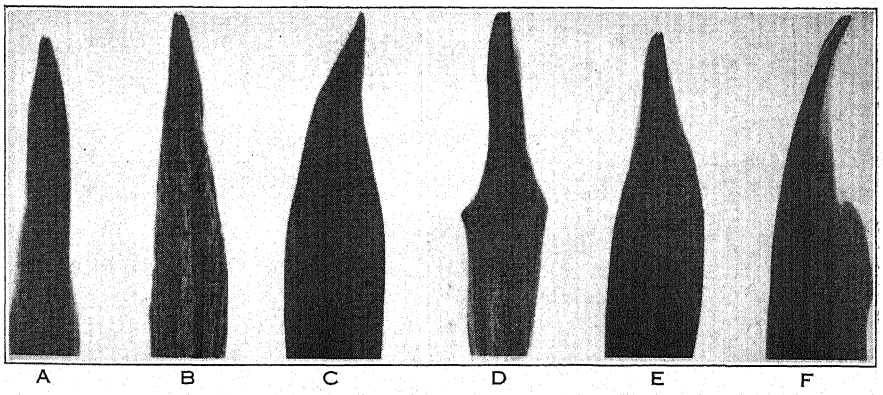


FIG. 8. Symptom expression of mottle viruses in leaves of Clara Butt tulip: A, VCM; B, CM; C, LT; D, TV1; E, TV2, in comparison with (F) control. A, B, C, E transferred by *Myzus persicae,* D transferred mechanically. Photographed by transmitted light.

but the *L. tigrinum* test was not then in use. The reactions of these 3 test plants indicate the presence of CM in Easter lily from several sources including occasional plants from Oregon, in some stocks of *L. candidum* from Oregon, in one *L. sargentiae* Wilson from Maryland, and in one *L. superbum* L. from New York. Reactions characteristic of LT were found for cultures from *L. auratum* Lindl. of Japanese origin, from some *L. tigrinum, L.*

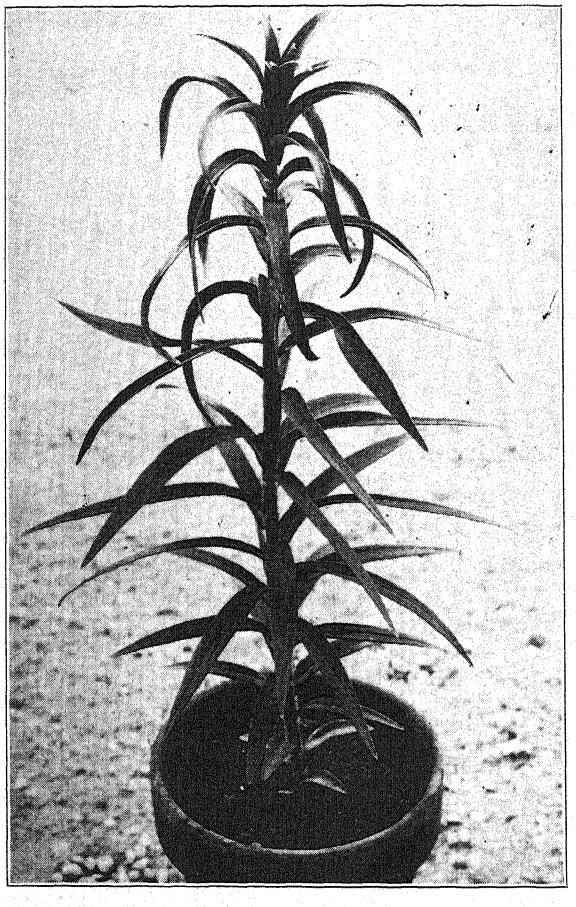


FIG. 9. Initial symptoms of CM photographed 15 days after inoculation into previously healthy *Lilium tigrinum* (in 6-inch pot). Gray etch in leaves immediately above those showing rubbing injury.

candidum, and *L. superbum* plants from New York, and from some Easter lilies from Oregon. Some isolates fail to fit the categories of table 1, which was prepared to distinguish the 5 cultures herein discussed, but which is not intended as a key to all mottle viruses affecting lily and tulip.

HOST RANGE OF THE EASTER LILY MOTTLE VIRUS (CM)

CM, which is readily transmissible by sap with the aid of carborundum, was tested by this method on a wide range of plants. No hosts were found

among 79 species of dicotyledonous plants representing 66 genera and 29 families, and including many of the common vegetable and ornamental plants. Subinoculations to *Lilium formosanum* from many of these plants were also uniformly negative. It is therefore believed unlikely that this virus is capable of infecting dicotyledonous plants.

A further survey of representative monocotyledons revealed no hosts for CM virus outside the Liliaceae, and few in this group. The following monocots were tested without result. AMARYLLIDACEAE: *Amaryllis* hybrids,

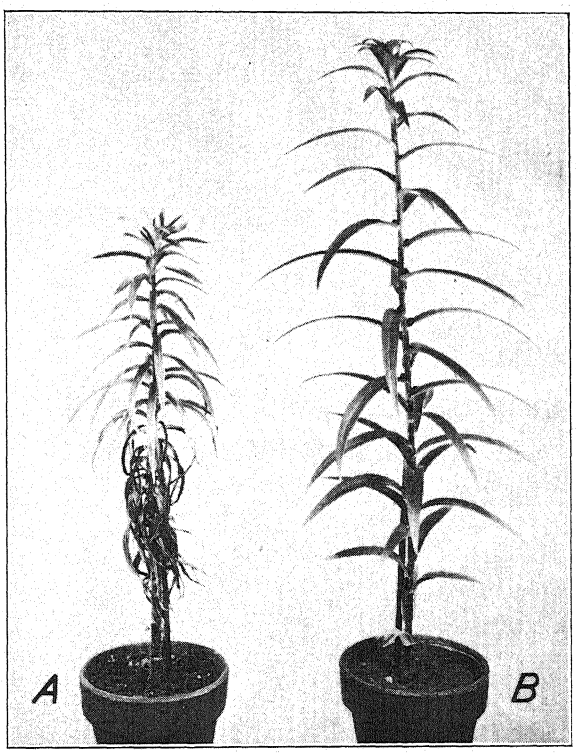


FIG. 10. Symptoms of CM in *Lilium tigrinum* (A) in comparison with control (B). (A) inoculated with LT in June, 1942, followed by CM in March, 1943. Photographed (in 5-inch pots) by Mead 45 days after CM was superimposed on LT. No protection was afforded by LT.

Narcissus pseudonarcissus L., *Zephyranthes* sp.; ARACEAE: *Philodendron* sp., *Zantedeschia aethiopica* Spreng.; CANNACEAE: *Canna generalis* Hort.; COMMELINACEAE: *Commelina coelestis* Willd., *C. communis* L., *Rhoeo discolor* Hance; DIOSCOREACEAE: *Dioscorea alata* L.; GRAMINEAE: *Avena sativa* L., *Saccharum officinarum* L., *Secale cereale* L., *Zea mays* L.; IRIDACEAE: *Moraea iridioides* L., *Tritonia crocata* Ker-Gawl.; LILIACEAE: *Agapanthus africanus* (L.) Hoffmg., *Allium cepa* L., *A. cernuum* Roth., *Aloe* sp., *Asparagus sprengleri* Regel, *Asphodeline lutea* Reichb., *Brodiaea uniflora* Baker, *Dracaena fragrans* Ker-Gawl., *D. sanderiana* Hort., *Gloriosa rothschildiana*

O'Brien, *G. superba* L., *Hyacinthus orientalis* L., *Kniphofia tucki* Baker, *Muscari polyanthum* Boiss., *Nothoscordum fragrans* Kunth., *Ophiopogon jaburan* Lodd., *Ornithogalum thyrsoides* Jacq., *Scilla hispanica* Mill., *Tricyrtis hirta* Hook., *Yucca flaccida* Haw.; MARANTACEAE: *Maranta bicolor* Ker.; MUSACEAE: *Musa cavendishii* Lamb.; ZINGIBERACEAE: *Hedychium coronarium* Koenig.

The established hosts of the CM virus are listed in table 2. In addition to species of *Lilium, Calochortus* sp., *Fritillaria pudica* Spreng., and *Zygadenus fremontii* Torr. have been shown to be susceptible. No symptoms were recognized in the two first named, but the virus was recovered. *Zygadenus fremontii* developed a fine green mottling resembling the pattern of iris

TABLE 2.—*Proved host plants of the Easter lily mottle virus (CM)*

Plants inoculated	No. of trials	Plants affected[a]	Symptoms	Subinoculation
Calochortus sp.	4	?/25	None	+
Fritillaria pudica Spreng.	2	?/10	"	+
Lilium dauricum Ker-Gawl.	1	2/2	Mottling, yellowing, death	
L. davidi Duchartre var. *Willmottiae*	1	1/4	Mottling	+
L. elegans Thunb.	1	3/3	Necrotic spots, yellowing, death	
L. formosanum Stapf	35	242/257	Mottling	+
L. leucanthum Baker	1	3/5	"	+
L. longiflorum Thunb.	28	195/275	"	+
L. superbum L.	1	2/5	"	+
L. tigrinum Ker-Gawl.	10	46/48	Necrotic spots, yellowing, death	+
L. umbellatum Hort.	1	3/3	Mottling, yellowing, death	
Tulipa gesneriana L.	39	264/428	Mottling, flower breaks	+
Zygadenus fremontii Torr.	3	5/11	Mottling	+

[a] Number plants affected over number exposed.

mosaic in bulbous iris. None of these plants were found naturally infected on receipt from plant dealers. Guterman (6) found *Fritillaria camtschatcensis* L. naturally affected with a mosaic only when grown in a nursery near affected lilies.

Comparatively few species of *Lilium* have been adequately tested for susceptibility because of the difficulty of obtaining vigorous stocks of known freedom from virus. Inoculation with CM resulted in mottling in seedlings of *L. davidi* var. *Willmottiae, L. leucanthum,* and *L. superbum,* and the virus was recovered from each of these. *Lilium dauricum, L. elegans, L. tigrinum* (Figs. 9, 10, 5, A), and *L. umbellatum* seedlings were killed by the virus. In parallel tests mechanical inoculation produced no infection in *L. davidi, L. henryi* Baker, *L. pardalinum* Kellogg, or *L. regale* Wilson seedlings, and transfers of *Aphis gossypii* Glover led to no infection in *L. humboldti* Roezl. and Leicht. var. *magnificum* Purdy, or in *L. parvum* Kellogg. The virus was not recovered from these species, but single trials even under conditions

favorable for infection of other species are not considered conclusive. As stated above, the CM type has been recovered from naturally affected *L. candidum*. It is to be expected that many other species of *Lilium* will prove susceptible, but this is the complete host list thus far proved for the CM virus.

VCM is mechanically transmissible to and from the test species listed in table 1. It has been so transmitted to *Zygadenus fremontii,* inducing symptoms indistinguishable from those of CM, but failed to infect *Calochortus* sp. in a single trial. Other host species of CM (Table 2) have not been inoculated with VCM. This virus alone of the group discussed herein has been transmitted to *Ornithogalum thyrsoides* by *Myzus persicae* (Fig. 2). Mechanical transfer from Easter lily to *Ornithogalum* failed, but VCM established in *Ornithogalum* by *M. persicae* was mechanically transferred to *Ornithogalum,* to Easter lily, and to *L. formosanum. Myzus persicae,* an efficient vector of VCM, failed to transfer this virus from Easter lily to Imperator iris (*Iris filifolia* Boiss.), to *Allium cernuum* Roth., to California Red onion, to *Allium fistulosum* L., or to *A. porrum* L. The known host range of VCM thus coincides with that of CM with 2 discrepancies, namely, the failure of VCM to infect *Calochortus* in a single trial, and the positive infection of *Ornithogalum thyrsoides.*

LT failed to infect either *Zygadenus fremontii* or *Calochortus* sp. in single trials. It may occur in additional species of lilies in nature, but only the species listed in table 1 have been experimentally infected. Similarly TV1 and TV2 have been studied here only in the 4 test plants listed in table 1. McWhorter (13) reports negative tests on onion, iris, narcissus, *Brodiaea,* and *Camassia.* The 2 tulip viruses have proved difficult to transmit from *Lilium formosanum* to Easter lily by sap, but this transfer has been proved by return inoculations from some of these symptomless Easter lilies to *L. formosanum.*

PROPERTIES OF THE EASTER LILY MOTTLE VIRUS (CM)

Preliminary data on the physical properties of CM were collected soon after this virus was recognized. It was soon evident that *Lilium formosanum* was a favorable source and test plant for such studies, but heavy demands on available stocks of this species as a test subject for subinoculation and indexing made it impracticable to complete property studies at once. Some determinations were therefore made with Easter lily as the source plant and Clara Butt tulips as test subjects. Sap for such tests was extracted in mortars and strained through cloth but not further cleared or purified. In thermal inactivation tests, 2-cc. samples of undiluted juice were pipetted into standard test tubes sold for this purpose, and heated in a De-Kotinsky water bath. In aging experiments undiluted juice was stored at 18° C. in open containers and 2-cc. samples tested for activity at stated intervals. For drying tests 5-cm. squares of cheesecloth were saturated with freshly extracted juice and suspended in shade at room temperature. After

stated intervals these squares were soaked in 10 cc. of tap water and used as inoculation pads.

Data on properties of CM virus are shown in table 3. The thermal inactivation point apparently lies between 60° and 65° C. and the dilution end point between 10^{-3} and 10^{-4}. The virus withstood aging 1 day but not 2 days, and was inactive after drying 1 day on cloth squares. Consistently lower tolerances were found when mottled Easter lily seedlings served as source of virus and forced Clara Butt tulips as test plants, indicating that this combination of species is poorly adapted to property studies. Norwood Creole Easter lily, a symptomless clon carrying the CM virus as shown by the reaction of tulip, *Lilium formosanum,* and *L. tigrinum,* yielded even lower readings, suggesting that the concentration of CM in this clon may be very low.

PROPERTIES OF FIVE VIRUSES OF THE TULIP GROUP

Parallel determination of properties of the 5 viruses were made in *Lilium formosanum,* the same species serving as source of virus. In general the source material was taken from plants about 3 weeks after inoculation when symptoms were well expressed and abundant leaf material was available. The procedures were as described above. In each experiment the source material, test plants, and manipulations were as uniform as possible for the 5 viruses compared. Each determination was duplicated, the results of the 2 separate trials being listed together in table 4 for convenience of comparison.

Thermal inactivation points between 60° and 65° C. were found for VCM, CM, and TV1, the 3 viruses that induce color removing or bleaching breaks in tulip, while points between 55° and 60° C. were found for LT and TV2, the viruses that induce color-adding, or intensifying, breaks in tulips. These data are consistent, and also in good agreement with the previous data for CM (Table 3); but McWhorter (13), who determined thermal inactivation points for TV1 and TV2 by inoculation from tulip to tulip, reports end points between 65° and 70° C. for both viruses with the comment "we have been unable to distinguish or separate the 2 viruses on a physical property basis."

The dilution end points found are less consistent than the thermal inactivation points. The differences between duplicate determinations on a single virus are as large as those between different viruses in a given test, except that LT yielded uniformly low end points. Dilution is the best index of concentration available for these viruses, and variable results such as these may indicate wide differences in concentration between samples assumed to be comparable. Such differences may be expected to affect other property determinations. According to these data the dilution end point of TV1 is beyond 10^{-4}, that of VCM between 10^{-4} and 10^{-5}, that of CM between $10^{-3.5}$ and 10^{-4}, that of TV2 between 10^{-3} and $10^{-3.5}$, and that of LT between 10^{-1} and 10^{-2}. Again it should be noted that McWhorter (13) found no differ-

TABLE 3.—*Properties of the Easter lily mottle virus (CM)*

Source of virus	Test species	Thermal inactivation point							
		Temperature, °C. during 10 min. exposure							
		Unheated	50°	55°	60°	65°	70°	75°	80°
L. formosanum	*L. formosanum*	10/10[a]	9/10	10/10	7/10	0/10	0/10	0/10	0/10
" "	" "	10/10	10/10	10/10	0/10	0/10	0/10	0/10	.....
" "	" "	10/10	10/10	10/10	9/10	0/10	0/10	0/10	.....
Easter lily sdlg.	Clara Butt tulip	13/15	10/15	5/14	0/13	0/15	0/15	.....	.....
Norwood Easter lily	" " "	4/15	1/15	0/15	0/15	0/15	0/15	.....	.....
		Dilution end point							
		Ratio of dilution of extracted sap							
		1/10	1/30	1/100	1/300	1/1,000	1/3,000	1/10,000	1/100,000	1/1,000,000
L. formosanum	*L. formosanum*	10/10[a]	.....	10/10	.....	10/10	.....	0/10	0/10	0/10
" "	" "	10/10	.....	10/10	.....	9/10	.....	0/10	0/10	0/10
Easter lily sdlg.	Clara Butt tulip	6/15	0/15	2/13	0/13	0/15	0/14	0/14	.....	.....
Norwood Easter lily	" " "	1/14	0/15	0/15	0/15	0/14	0/15	0/15	.....	.....
		Tolerance of aging							
		Number of days extracted sap was aged at 18° C.							
		0	1	2	3	4	5	6	10
L. formosanum	*L. formosanum*	.....	9/10	0/10	0/10	0/10	.....	0/10	.....
Easter lily sdlg.	Clara Butt tulip	10/14[a]	1/15	0/15	0/15	.....	0/15	.....	0/14
Norwood Easter lily	" " "	0/15	0/15	0/14	0/13	.....	0/14	.....	0/14
		Tolerance of drying							
		Number of days dried on cloth							
		0	1	2	3	5	10		
Easter lily sdlg.	Clara Butt tulip	13/15[a]	0/15	0/15	0/15	0/15	0/15	.....	.....
Norwood Easter lily	" " "	0/14	0/9	0/14	0/10	0/15	0/15	.....	.....

[a] Number of plants infected over number exposed.

TABLE 4.—*Properties of 5 viruses of the tulip group, as determined in sap of Lilium formosanum tested on L. formosanum*

Virus	Test No.	Thermal inactivation point					
		Temperature, ° C. during 10 min. exposure					
		Unheated	50°	55°	60°	65°	70°
VCM	3	5[a]	5	5	2	0	0
	8	5[a]	4	5	4	0	0
CM	3	5	5	5	5	0	0
	8	5	5	5	5	0	0
LT	3	2	5	1	0	0	0
	8	5	4	1	0	0	0
TV1	3	5	5	5	5	0	0
	8	4	5	2	0	0	0
TV2	3	4	5	4	0	0	0
	8	4	4	4	0	0	0

		Dilution end point					
		Ratio of dilution of extracted sap					
		1/10	1/100	1/1,000	1/3,000	1/10,000	1/100,000
VCM	4	7[b]	6	2	0	0	.....
	7	8[b]	8	4	3	1	0
CM	4	8	7	1	2	0	.....
	7	8	8	4	3	0	0
LT	4	7	0	0	0	0	0
	7	7	0	0	0	0	0
TV1	4	8	8	3	3	3	.....
	7	8	6	4	0	0	0
TV2	4	5	1	1	0	0	.....
	7	8	5	0	0	0	0

		Tolerance of aging						
		Number of days extracted sap was aged at 18° C.						
		0	1	2	3	4	5	6
VCM	2	10[c]	9	9	.....	.....	2	.....
	5	5[a]	5	5	4	5	4	3
CM	2	10	10	10	.....	.....	1	.....
	5	5	5	4	3	5	4	0
LT	2	9	6	3	.....	.....	0	.....
	5	5	5	3	1	3	0	0
TV1	2	10	9	8	.....	.....	0	.....
	5	5	5	5	4	4	5	4
TV2	2	10	0	0	.....	.....	0	.....
	5	5	5	1	2	3	0	0

		Tolerance of drying						
		Number of hours dried on cloth						
		0	2	4	6	8	24	32
VCM	1	10[c]	.....	0	.....	0	0	0
	6	5[a]	3	1	2	.....	.....	.....
CM	1	10	.....	0	.....	0	0	0
	6	5	2	2	0	0	0	0
LT	1	8	.....	0	.....	0	0	0
	6	3	0	0	0	.....	.....	.....
TV1	1	10	.....	0	.....	0	0	0
	6	5	4	2	0	.....	.....	.....
TV2	1	8	.....	0	.....	0	0	0
	6	5	0	0	0	.....	.....	.....

[a] Number of plants infected of 5 exposed.
[b] Number of plants infected of 8 exposed.
[c] Number of plants infected of 10 exposed.

ences in dilution properties between TV1 and TV2, reporting both infectious at dilutions of 10^{-5}.

The tests on tolerance of aging were not continued beyond 6 days, as earlier determinations for CM (Table 3) indicated an end point between 1 and 2 days. These tests failed to establish end points for VCM and TV1, both of which were active after 6 days' aging. The CM virus was active after 5 days but not after 6 days, and both LT and TV2 produced infection after 4 days' but not after 5 days' aging. Guterman (6) found the virus causing mottling of *Lilium speciosum* Thunb. var. *rubrum* Masters to be infectious to this species after aging 2 hours but not after $2\frac{1}{2}$ hours or more.

In the first experiment on tolerance to drying on cloth squares none of the 5 viruses were active after 4 hours or more, but in a duplicate experiment VCM was found infectious after 6 hours' drying, CM and TV1 after 4 hours', but not after 6 hours' drying, while TV1 and TV2 failed to withstand 2 hours' drying under these conditions. McWhorter (13) reports

TABLE 5.—*End points determined for 4 physical properties of mottle viruses*

Virus	Thermal inactivation (°C.)	Dilution	Aging (days)	Drying (hours)
VCM	60	10^{-4}	6+	6
TV1	60	10^{-4}	6+	4
CM	60	$10^{-3.5}$	5	4
TV2	55	10^{-3}	4	0
LT	55	10^{-1}	4	0

TV1 and TV2 still active after 11 days' drying in tulip leaves. Guterman (6) found no virus capable of infecting *Lilium speciosum rubrum* in leaves of this species or leaves of *L. auratum* or *L. longiflorum* that had dried completely between blotters.

The experimentally determined end points for the 5 viruses are collected for convenient reference in table 5. Although the differences between extremes may be considered sufficiently great to separate distinct viruses, the 5 viruses obviously form a related series. The 3 viruses VCM, TV1, and CM, which are the ones causing color-removing breaks in tulips, show close agreement in properties, with tolerances higher than those of the 2 color-adding viruses TV2 and LT.

VECTOR RELATIONS OF THE MOTTLE VIRUSES

Only *Aphis gossypii* had been established as a vector of lily viruses when the present study was undertaken. Ogilvie (17) found this species to be the sole vector of lily-rosette virus. Guterman (6) found that *A. gossypii* transmitted a virus from mosaic *Lilium speciosum rubrum* to *L. speciosum rubrum* (10/10, 8/10), to *L. longiflorum formosum* (6/10, 13/20), and to *L. auratum* (4/5); from *L. auratum* to *L. longiflorum formosum* (3/5) and to *L. speciosum rubrum* (6/8); and from *L. longiflorum formosum* to *L.*

speciosum rubrum (4/5). He also showed each of 4 stages of this vector to be capable of transmitting the virus, and proved a single aphid could transmit it. In similar trials Guterman obtained negative results with *A. ogilvei* Theob., *A. fabae* Scop., *Macrosiphum solanifolii* Ashm., *Myzus circumflexus* Buckt., and *M. persicae*. Guterman's results cannot be satisfactorily correlated with the writers' at present, because he dealt largely with the virus causing stripe mottling in *L. speciosum* and *L. auratum*, species not yet studied in detail by the present writers.

The demonstration (2, 12, 14) that certain lily viruses could induce breaks in tulips closely similar to those brought about by the tulip-breaking viruses in nature suggested that these lily viruses might be transmissible by the vectors of tulip-breaking viruses, *Macrosiphum solanifolii* and *Myzus persicae* (4, 9, 10, 11). A study of vectors of lily viruses, with particular emphasis on Easter lily viruses, was therefore undertaken in 1939.

Methods

Plants were moved to a separate insectary greenhouse for aphid inoculation trials. In the early experiments non-viruliferous aphids were colonized on infected source plants and allowed to feed for several days or longer before they were transferred to healthy test plants. In later tests such non-viruliferous aphids were transferred from favorable food plants to Petri dishes containing pieces of leaves, stems, or flowers from the diseased source plant and allowed to feed for 1 to 3 hours. When such source material was held at about 18° C. the aphids settled down and fed readily but wandered about and congregated on the top or sides of the dish when temperatures were higher. These pieces of source plant material with the aphids in place were then transferred by forceps to pieces of paper resting on leaves of the healthy plants being inoculated, thus avoiding contact of the diseased material with the healthy plants. Under these conditions the aphids moved freely to the leaves of the test plants as the source material withered. After 1 to 5 days' feeding the survivors were destroyed by pyrethrum or rotenone sprays, and the plants were returned to the aphid-free greenhouse for observation.

Aphis gossypii extricates its mouth parts from the plant tissue with greater difficulty than do the other species investigated and was therefore transferred by brush less readily. It may be that lower percentages of disease transmission by this species were due in part to injury of individuals during handling. This did not apply to *Macrosiphum solanifolii*, however, which is readily transferred by brush.

Experimental Results

The data in table 6 show that *Macrosiphum solanifolii* and *Myzus persicae*, as well as *Aphis gossypii* are vectors of CM. No transmission was effected by *A. fabae*, *Macrosiphum lilii* Monell, *Myzus circumflexus*, or *Myzus convolvuli* Kalt. under comparable conditions. Negative results were

confirmed by mechanical subinoculation to *Lilium formosanum*. It is of interest that *Macrosiphum lilii, Myzus convolvuli,* and *Myzus circumflexus* colonize readily on Easter lilies but do not transmit this virus, while *Aphis gossypii* is the only vector species with similar host preferences. *Macrosiphum solanifolii* and *Myzus persicae* will colonize maturing healthy plants but die within a few days on younger growth.

Aphis gossypii proved to be comparatively inefficient as a vector of CM. Its failure to transmit from Easter lily to *Lilium tigrinum* is particularly surprising inasmuch as this transfer is easily accomplished mechanically. In view of this failure little weight is placed on the negative results on *L. humboldtii magnificum* and *L. parvum*. *Macrosiphum solanifolii* was also inefficient in these tests, and even *Myzus persicae* gave low proportions of transfer in earlier trials; but in later tests, with use of the Petri-dish method and short feeding periods on source plant tissue, it was highly efficient. This species was utilized in comparative trials with VCM, TV1, TV2, and LT. If an incubation period of these viruses occurs in *Myzus persicae* it is less than 1 day's duration since successful transfers were obtained within that period.

VCM was readily transmitted by *Myzus persicae* (Table 7) from Easter lily to Easter lily, *Lilium tigrinum,* and tulip, and somewhat less readily to *Ornithogalum thyrsoides*. When established in *Ornithogalum,* this virus was readily transferred to *Ornithogalum,* Easter lily, and tulip. Symptoms induced in *L. tigrinum* on transfer of VCM by *M. persicae* agreed fully with those produced mechanically, including initial gray surface flecking with subsequent progressive yellowing and death. As already noted, *M. persicae* in parallel trials failed to transmit VCM to onion, *Allium cernuum, A. fistulosum, A. porrum,* or *Iris filifolia*. *Myzus circumflexus* from similar source material failed to transmit VCM to tulip, Easter lily, or *Ornithogalum;* no evidence has been found that this species can transmit either CM or VCM.

In parallel trials (Table 7) *Myzus persicae* transferred TV1, TV2, and LT to tulip with high efficiency, but failed to transfer any of these to *Ornithogalum thyrsoides*. Symptoms induced in tulips on transfer of the viruses by *M. persicae* agreed closely with those produced on mechanical inoculation with the same viruses. Symptoms tended to appear earlier following aphid transfer. Clara Butt tulips, exposed to aphids soon after the leaves appeared above the soil, developed recognizable leaf symptoms 11 days after introduction of TV1 in one test. Other viruses producing strong leaf symptoms (CM, VCM) sometimes produced symptoms in 15 or 16 days, but TV2 and LT, which mottle tulip leaves weakly or not at all, were not positively diagnosed until the tulips flowered, 30 to 38 days or longer after inoculation.

In a few additional trials (Table 8) commercial Rembrandt tulips, carrying TV1 and TV2 in mixture according to McWhorter's (15) interpretation, served as source plants and *Lilium formosanum* as test plants. The estab-

TABLE 6.—*Transmission of lily mottle virus (CM) by aphids*

Vector species	Source of virus	Plants exposed	No. tests	Plants affected[a]	Minimum incubation period (days)	Symptoms
Aphis fabae	*L. formosanum*	*L. formosanum*	1	0/2	.....	None
A. gossypii	Easter lily	Easter lily sdlgs.	18	5/193	10	Mottle
" "	" "	*L. formosanum*	6	9/26	13	"
" "	" "	Clara Butt tulip	5	10/28	36	Flower break
" "	" "	*L. tigrinum*	2	0/15	.....	None
" "	" "	*L. humboldtii magnificum*	1	0/15	.....	"
" "	" "	*L. parvum*	1	0/5	.....	"
" "	*L. formosanum*	Easter lily sdlgs.	1	0/15	.....	"
" "	*L. candidum*	" " "	2	9/20	29	Mottle
" "	*L. formosanum*	*L. formosanum*	18	7/88	17	"
Macrosiphum lilii	Easter lily	" "	3	0/20	.....	None
" "	*L. formosanum*	" "	7	0/37	.....	"
M. solanifolii	Easter lily	Easter lily sdlgs.	1	1/2	20	Mottle
" "	" "	Clara Butt tulip	2	1/20	31	Flower break
Myzus circumflexus	" "	Easter lily sdlgs.	4	0/40	.....	None
M. convolvuli	" "	" " "	4	0/11	.....	"
M. persicae	" "	Clara Butt tulip	4	29/68	15	Flower break
" "	" "	Easter lily sdlgs.	6	12/39	16	Mottle
" "	" "	*Ornithogalum thyrsoides*	1	0/5	.....	None
" "	*L. formosanum*	Clara Butt tulip	1	5/5	15	Flower break

[a] Number of plants infected over number exposed.

lished vectors of tulip breaking (4, 9, 10, 11), *Macrosiphum solanifolii* and *Myzus persicae,* effected transfer of viruses as expected, and 2 species that failed to carry lily mottle viruses, *Macrosiphum lilii* and *Myzus circumflexus,* also failed to carry tulip-breaking viruses. *Aphis gossypii,* an inef-

TABLE 7.—*Transmission of the mottle viruses VCM, TV1, TV2, and LT by Myzus persicae*

Source of virus	Plants exposed	No. tests	Plants affected[a]	Minimum incubation period (days)	Symptoms
Virulent mottle virus (VCM)					
Easter lily	Easter lily sdlg.	15	67/93	11	Mottle, leaf distortion
" "	*L. tigrinum*	1	5/5	8	Gray flecks, yellowing, death
" "	Clara Butt tulip	4	29/35	16	Mottle, flower-break
" "	*Ornithogalum thyrsoides*	4	9/20	22	Mottle
Ornithogalum thyrsoides	"	1	4/5	26	"
"	Easter lily sdlg.	1	4/5	14	Mottle, leaf distortion
"	Clara Butt tulip	1	5/5	15	Mottle, flower-break
L. formosanum	" " "	1	5/5	15	" "
Tulip virus 1 (TV1)					
L. formosanum	Clara Butt tulip	2	8/8	11	Mottle, flower-break
" "	*Ornithogalum thyrsoides*	1	0/5	.....	None
Tulip virus 2 (TV2)					
L. formosanum	Clara Butt tulip	1	5/5	15	Mottle, flower-break
Clara Butt tulip	" " "	1	4/4	30	" "
L. formosanum	*Ornithogalum thyrsoides*	1	0/5	.....	None
Latent virus (LT)					
L. formosanum	Clara Butt tulip	1	5/5	38	Mottle, flower-break
" "	*Ornithogalum thyrsoides*	1	0/5	.....	None

[a] Number of plants infected over number exposed.

ficient vector of lily-mottle viruses, effected a single transfer of tulip-breaking virus. *Aphis fabae,* little tested by workers on either tulips or lilies, proved very efficient in transmitting tulip-breaking viruses. The last 2 species, *A. fabae* and *A. gossypii* are here recorded for the first time as vectors of tulip-breaking viruses.

TABLE 8.—*Transmission of virus from commercial Rembrandt tulips to Lilium formosanum by aphids*

Vector species	Source of virus	Plants exposed	No. tests	Plants affected[a]	Minimum incubation period (days)	Symptoms
Aphis fabae	Rembrandt tulip	*L. formosanum*	2	8/9	13	Mottle
A. gossypii	"	"	1	1/8	19	"
Macrosiphum lilii	"	"	1	0/8	.....	None
M. solanifolii	"	"	1	4/8	19	Mottle
Myzus circumflexus	"	"	1	0/8	.....	None
M. persicae	"	"	1	6/8	19	Mottle

[a] Number of plants affected over number exposed.

TABLE 9.—*Protective effects of some lily mottle viruses against other viruses of this group*

Mottle virus first introduced	Date of first inoculation	Method of determining presence of first virus	Mottle virus introduced second	Date of second inoculation	Symptoms expressed	Protective effect
CM (?) (masked) in Croft Easter lily	Natural infection	Indexing	CM	(1938) Dec. 8	Trace of mottling	Essentially complete
CM (masked) in Florida lily	"	"	CM	Dec. 8	None	Complete
CM (masked) in Norwood Easter lily	"	"	CM	(1940) Feb. 10	"	"
"	"	"	VCM	(1943) Feb. 17	"	"
"	"	"	VCM[a]	Mar. 9	"	"
CM in Easter lily sdlg.	(1943) Feb. 17	Symptoms	VCM	Mar. 24	"	"
LT (masked) in Easter lily sdlg.	Feb. 17	Indexing	VCM	Mar. 24	Mottling, distortion	None
LT in *L. tigrinum*	(1942) June 4	"	CM	Mar. 24	Etch, yellowing, death	"
TV1 (masked) in Easter lily sdlg.	(1943) Mar. 24	"	VCM	Apr. 26	Mottling, distortion	"
TV2 (masked) in Easter lily sdlg.	Mar. 24	"	VCM	Apr. 26	"	"

[a] Second virus added by *Myzus persicae* here, in all other tests by mechanical methods.

PROTECTIVE ACTION OF CERTAIN MOTTLE VIRUSES AGAINST CM AND VCM

Inasmuch as CM and VCM produce symptoms in Easter lilies and TV1, TV2, and LT do not, the Easter lily may serve as a test plant for protective action of certain of these viruses against CM or against VCM. Also *Lilium tigrinum,* which is mildly affected by LT, TV1, or TV2, but killed by CM or VCM, has similar advantages for protective tests. Tests of this type that have been completed are summarized in table 9.

The CM symptoms could not be induced in certain commercial Easter lily stocks (Croft, Florida, Norwood Creole) naturally infected with this

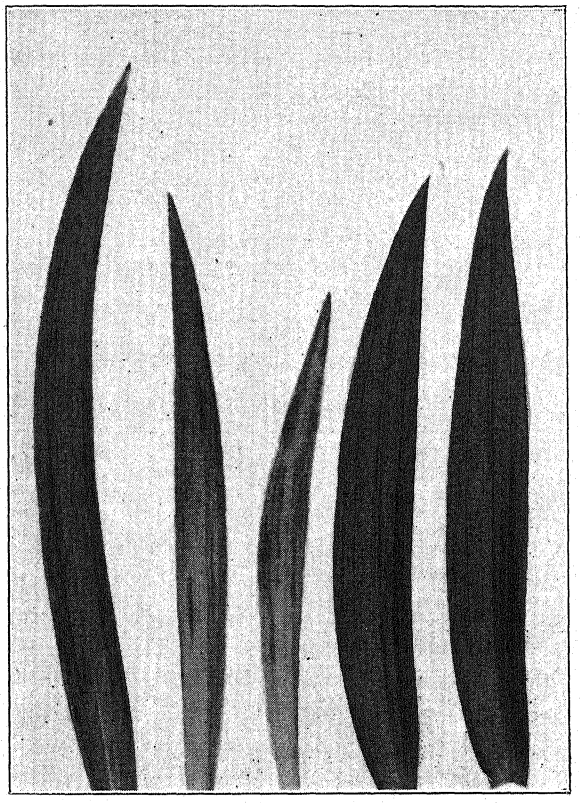


FIG. 11. VCM superimposed on TV1 in 3 leaves at left with, right, 2 leaves TV1 controls. TV1 introduced Mar. 24, 1943; presence established by indexing. VCM introduced April 26, 1943. Photographed June 9, 1943, by Mead. VCM was similarly superimposed on TV2 and LT in separate trials but not on CM.

or a similar virus but showing no symptoms. Similarly VCM failed to induce symptoms in Norwood Creoles when introduced mechanically or by *Myzus persicae,* and VCM failed to develop further distinctive symptoms when superimposed on CM experimentally produced and expressing the usual mottling symptoms in Easter lily seedlings. In contrast VCM showed full expression of symptoms when superimposed on LT, TV1 (Fig. 11), or TV2, previously established but symptomless in Easter lily seedlings. In a

single test in *Lilium tigrinum*, LT established in the previous season of growth showed no protective action against CM when this virus was superimposed (Fig. 10).

The data thus far available on protective action suggest that CM and VCM are closely allied, but distinguish these from TV1, TV2, and LT. This separation is in agreement with the test reactions in Easter lily and in *Lilium tigrinum* (Table 1) but at variance with the test reactions in tulip and with the property values (Table 5) which place TV1 in the group with CM and VCM.

NEGATIVE EVIDENCE ON SEED TRANSMISSION OF LILY VIRUSES

Inasmuch as masking of CM is so common in Easter lilies as to make roguing impracticable, seedlings appear to offer the only means of producing virus-free stocks. LT, and probably other viruses of the mottle group, presents similar problems in various garden species. Evidence that these viruses are not seed-borne is therefore basic to any program of producing virus-free lilies.

TABLE 10.—*Number of seedlings grown from mosaic Easter lily plants and showing no evidence of seed-borne virus*

Symptoms in ♀ parent	Symptoms in ♂ parent				
	Mottling	Necrotic fleck	None	Unknown	Totals
Mottling	923 (23)[a]	588 (9)	2748 (56)	207 (8)	4466 (96)
Necrotic fleck	743 (16)	233 (34)	1867 (34)	69 (3)	2912 (57)
None	602 (18)	870 (16)	1326 (29)	2736 (53)	5534 (116)
Totals	2268 (57)	1691 (29)	5941 (119)	3012 (64)	12912 (269)

[a] Total number of seedlings, with number of progenies in parentheses.

Guterman's (6) excellent data on seed transmission, which have not heretofore been made available to most workers, are worth presenting here. He detected no mosaic symptoms in the following numbers of seedlings from mosaic parent species: *Lilium candidum* 91, *L. davuricum* 878, *L. henryi* 74, *L. longiflorum* 84, *L. regale* 487, *L. sargentiae* 270, *L. speciosum rubrum* 252, *L. tenuifolium* 253, *L. tenuifolium* var. Golden Gleam 89, *L. umbellatum* 139. He concluded that the virus of lily mosaic is not transmitted in the seed.

The writers have data (Table 10) on seedlings of *Lilium longiflorum* only. In connection with breeding research on this species, seeds of 269 progenies were sown in greenhouses in September, 1936, grown continuously under glass to the 3-inch-pot stage, and inspected for virus symptoms until they were dried off in July, 1937. The virus symptoms expressed in each parent plant were recorded except for open-pollinated progenies of which the male parents were unknown. Extensive indexing in tulip and *L. formosanum* indicates that practically all commercial Easter lilies carry a mottle virus even if symptomless, and that necrotic fleck in commercial

Easter lilies is uniformly accompanied by a virus of this group. Accordingly, all of the 12,912 seedlings (Table 10) produced from commercial Easter lily parents would appear to contribute valid evidence against seed carriage of viruses of the mottle group in this species. The possibility of masking in these test seedlings themselves has been considered, but indexing of numbers of random samples of these on *L. formosanum* and tobacco has yielded no evidence that either mottle viruses or cucumber-mosaic virus can be seed-borne but masked. It may therefore be fairly stated that no evidence is known that any lily virus is seed-borne.

DISCUSSION AND CONCLUSIONS

Although the lily viruses VCM, CM, and LT are those of chief interest in the present study, the tulip viruses TV1 and TV2 are intimately concerned in the problems of classification and nomenclature. Smith (19) included TV1 and TV2 under the name Tulipa Virus 1 (Cayley), and, accepting Atanasoff's (1) account, listed hyacinth mosaic and narcissus mosaic as caused by the same virus. Holmes (8) erected the species *Marmor tulipae* for TV2, and *Marmor mite* for TV1 and Lily-latent virus, combining the two last named on the strength of the limited evidence published by McWhorter (14) up to that time. It is now apparent that the symptoms McWhorter assigned to his latent virus in tulips resembling those of TV1, although characteristic of viruses from the source species *Lilium candidum* and *L. longiflorum,* do not fit our LT from *L. tigrinum* which has much more in common with TV2. In earlier accounts of the "strong mottle of Easter lily" Brierley (2, 3) did not attempt to class this virus farther than "the tulip group" of viruses, namely the group studied herein. The virulent mottle virus VCM is reported for the first time in the present paper.

It is evident that the data presented above would serve to set up and name 2, 3, 4, or even 5 separate virus entities, without violating commonly accepted standards for differentiating mosaic viruses. Thus symptoms in Easter lily and *Lilium tigrinum* (Table 1) and protection reactions in these 2 species, place the 5 cultures in 2 groups: VCM–CM and TV1–TV2–LT. But the tulip reactions, and the properties as determined in *L. formosanum* (Table 5), serve to group them differently: VCM–CM–TV1 and TV2–LT, while the reaction of *Ornithogalum* tends to set VCM apart as a distinct entity. Thus VCM and CM form a natural group, with one host reaction difference (in *Ornithogalum*) and symptom differences in Easter lily that are of the order expected of strains. Similarly TV2 and LT appear closely allied, with strain differences in symptom expression in *L. formosanum* and *L. tigrinum.* It is TV1 that fails to conform to either of these groups, showing affinity to the first in tulip reaction and in properties, but resembling the second group in Easter lily reaction and in protection relations.

However, the writers are reluctant to add further to the confusion in nomenclature, and prefer to use a single binomial for the group, indicating the 5 members studied as sub-species or strains. This requires amending

Marmor tulipae Holmes (8) to include color-removing, as well as color-adding, break symptoms in tulips. Tulip-breaking virus, so designated, has a host range in Liliaceae of *Lilium formosanum, L. longiflorum, L. tigrinum,* and *Tulipa gesneriana,* with many other species of lilies presumably susceptible, and a few other hosts known for specific strains. Symptoms vary with the strains, as shown in table 1. Transmission by sap and by *Aphis gossypii, Macrosiphum solanifolii,* and *Myzus persicae,* but not through seed, characterizes *Marmor tulipae* thus defined. *Anuraphis tulipae* Boyer (11), and *Aphis fabae* (herein) are recorded as vectors of tulip color-adding and tulip color-removing strains. Properties as determined in *L. formosanum* also vary with the strain (Table 5) : thermal inactivation point 60° to 65° C. or lower; dilution end-point 10^{-4}, or at less dilution; active in expressed juice after 6 days (4 days), and after drying on cloth for 6 hours (0 hours). This conception of *Marmor tulipae* is in agreement with Tulipa virus 1 (Cayley) in Smith (19) except that the reported hosts *Hyacinthus* and *Narcissus* must be omitted.

The following sub-species and strains of tulip-breaking virus may be described from the data provided in this paper:

Tulip-color-removing sub-species (the type sub-species). Predominantly color-removing breaks in tulip flowers; symptomless in Easter lily; green mottling soon masked in *Lilium tigrinum;* sparse and fine green island mottling in *L. formosanum;* thermal inactivation point 60° to 65° C.; dilution 10^{-4}; withstands aging 4 days, drying 4 hours; fails to protect against virulent mottle virus (VCM) in Easter lily.

Synonyms: Tulip color removing strain McWhorter (15) part of *Marmor mite* Holmes (8).

Tulip-color-adding sub-species. Predominantly color-adding breaks in tulip flowers; symptomless in Easter lily; non-fatal effects in *Lilium tigrinum;* mottling without yellowing or dwarfing in *L. formosanum;* thermal inactivation point 55° to 60° C.; dilution 10^{-1} to 10^{-3}; withstands aging 4 days; fails to withstand drying 2 hours; fails to protect against virulent mottle virus (VCM) in Easter lily.

Strains: TV2, tulip-color-adding strain McWhorter (15), *Marmor tulipae* (8), coarse dark and light green mottling in *Lilium formosanum,* gray surface etch in *L. tigrinum;* LT (of this paper), part of lily latent virus McWhorter (14), part of *Marmor mite* Holmes (8), green island mottling in *L. formosanum,* green mottling soon masked in *L. tigrinum.*

Lily-mottle sub-species. Predominantly color-removing breaks in tulip flowers; mottling in Easter lily; gray surface etch followed by general yellowing and death in *Lilium tigrinum;* mottling, yellowing, and dwarfing in *L. formosanum.* Properties in *L. formosanum:* thermal inactivation point 60° to 65° C.; dilution $10^{-3.5}$ to 10^{-4}; withstands aging 5 days or more, drying 4 to 6 hours.

Strains: CM (coarse mottle), strong mottle of Easter lily (3), mottling without distortion in Easter lily; VCM (virulent mottle), leaf and flower distortions in Easter lily, mottling in *Ornithogalum thyrsoides.*

The rank assigned these sub-species and strains may be questioned by others who attach relatively greater importance to certain of the criteria employed. The writers consider host and vector relations of fundamental importance, and find the 5 cultures studied to be relatively uniform in these respects. No great weight is attached to minor differences in host range and properties, for we have found similar differences among recognized strains of cucumber-mosaic virus. Protection tests of the type reported here, namely attempts to superimpose a second systemic infection on a similar first infection, are not in themselves considered decisive as tests of relationship.

The rank properly assigned these cultures is also dependent on their inherent constancy. Although little direct evidence of inconstancy can be cited, there is some reason to believe that these types are too variable to be separated as valid species. VCM is assigned strain rank as a mutant of CM, but no other evidence of mutation of one type to another is available. However, the position of TV1 as a connecting link between the other two subspecies described here suggests definite continuity in the group as a whole. Also, TV1 and TV2, the types on which McWhorter (15) and Holmes (8) have erected separate species, occur in mixture in nature, and have been separated (15) only by sub-culturing from natural segregates. Of these, TV1 is difficult to maintain in tulip, tending to kill the affected plants (15). But neither TV1 nor TV2 is lethal to *Lilium formosanum,* and our data on properties (Table 4) suggest that TV1 may be separable from TV2 by its greater tolerance to aging or drying. Unless some such technique can be shown to separate entities producing distinctive break symptoms in tulips, it does not seem advisable to accord TV1 and TV2 more than sub-specific rank, if they indeed merit more than strain designation.

Lack of seed transmission and the limited host range of lily-mottle strains (VCM, CM, LT) show that spread of these viruses in nature must take place almost exclusively from lily to lily. *Calochortus, Fritillaria,* and *Zygadenus,* though susceptible to CM, like *Lilium* spp. from the wild, appear to be free from virus unless they have been contaminated from diseased lilies. Tulips are often affected with tulip-breaking virus in nature, and as these plants mature when many lilies are in active growth, some spread from tulips to lilies may be expected. The fact that the virus strains thus far detected in lilies are usually different from the tulip strains indicates, however, that such spread is uncommon. Lily seedlings adequately isolated from bulb-propagated lilies should remain virus free. Easter lily seedlings well isolated at Willard, North Carolina (1 season), and at Charleston, South Carolina (3 seasons), did remain virus free, as determined by symptoms and by indexing samples; but similar seedlings inadequately isolated elsewhere developed both mottle and necrotic fleck. Guterman (6) reached similar conclusions, his inoculations to and from lily revealing only one host, a *Fritillaria,* outside the genus *Lilium.* He adds that "a large number of cases have been under observation for periods of 3 and 4 years in which

healthy groups of lilies growing in gardens in various localities far removed from any mosaic plants of the same genus have remained healthy during this time even though the known insect vector of the virus was repeatedly observed feeding upon them.''

Although production of virus-free lilies from seed appears feasible, Easter lily seedlings are too variable for forcing use unless selected for this purpose. American growers seem unanimous in the conclusion that production of virus-free Easter lilies is not commercially practicable. Easter lilies force satisfactorily when affected with CM, but direct comparisons of the performance of virus-free and CM stocks of the same clon have not been made. This virus strain, or allied types, is apparently universal in commercial Easter lily stocks, but is so frequently masked that roguing is not feasible. The soundness of the decision to ignore mottle in commercial Easter lily production was thrown into question once more with the appearance of virulent mottle (VCM), which produces some cull plants. It became of immediate importance to learn whether VCM is a mutant from CM or a distinct virus. The evidence detailed above is believed adequate to show that it is a mutant of CM. Physical properties of the two agree closely (Table 5), no differences in vector relations are known, and but one additional host, *Ornithogalum thyrsoides,* is known for VCM. Protection tests (Table 9) indicate very close relationship, for VCM was not superimposed on CM by mechanical methods or by *Myzus persicae.* This last finding indicates that VCM cannot spread into Creole lilies, but that it arises spontaneously from the CM virus already present in these, for VCM is known to appear in Creole lilies under circumstances that simulate natural spread. The VCM type has been found to appear naturally only in stocks of CM history. In some transfers by *Myzus persicae* from Easter lilies of supposedly pure CM content to Easter lily seedlings, the VCM symptoms have appeared, suggesting that this vector picked up such mutant types not recognizable in the source plant by symptom expression.

If CM is to be ignored in commercial Easter lily production because it cannot be effectively rogued, the same logic requires that VCM be ignored also. If VCM arises spontaneously from CM, as our evidence indicates, roguing the plants that express VCM symptoms will not eliminate this virus. In our experience the incidence of VCM in forced Creole lilies is comparatively low, and, in affected plants the virulent symptoms are not always expressed at the top where they would result in cull blooms, so that the proportion of unsalables has not been high. As the recurrence of the virulent symptoms from year to year also appears to be low, VCM does not appear to be a serious menace in field production.

More or less seasonal waves of migrant aphids are evidently responsible in large part for the general prevalence of mottle viruses in lilies. Potatoes (*Solanum tuberosum* L.) and cruciferous crops (*Brassica* spp., etc.) are favored food plants of *Myzus persicae.* When such crops mature, or when they become heavily infested, the aphids develop winged migrants in

large numbers. These migrants carry no virus from potatoes or crucifers to lilies, and do not prefer lilies as food plants. When they encounter lilies, however, they do feed briefly on a great many individual plants, and thus prove highly efficient vectors of lily mottle viruses that are already present. Spread of lily-mottle virus by *M. persicae* under such circumstances is clearly recognizable in indicator species such as *Lilium formosanum.*

SUMMARY

Three mottle viruses from lilies, a latent type from *Lilium tigrinum* (LT), the strong mottle of Easter lily (CM), and a more virulent mutant from the latter (VCM), are compared with McWhorter's Tulip Viruses 1 and 2 in the test species Easter lily, *L. formosanum, L. tigrinum,* and tulip.

The host range of CM is found limited to Liliaceae, including *Calochortus* sp., *Fritillaria pudica,* tulips, and *Zygadenus fremontii,* in addition to several species of lilies. The known ranges of the other 4 viruses are similar except that the VCM strain infects also *Ornithogalum thyrsoides.*

Properties of the 5 viruses were determined in *Lilium formosanum.* The thermal inactivation points of VCM, CM, and TV1 were found to lie between 60° and 65° C.; those of TV2 and LT, between 55° and 60° C.

Two viruses, VCM and TV1, were active after dilution to 10^{-4}, CM at $10^{-3.5}$, TV2 at 10^{-3}, and LT at 10^{-1}. All 5 viruses were active after aging 4 days at 18° C., CM after 5 days, and VCM and TV1 after 6 days. VCM survived 6 hours' drying on cloth, CM and TV1 survived 4 hours, while TV2 and LT were inactive after drying 2 hours.

CM is transmitted by the aphids *Aphis gossypii, Macrosiphum solanifolii,* and *Myzus persicae,* but not by the common lily infesting species *Myzus circumflexus, M. convolvuli,* or *Macrosiphum lilii. Myzus persicae* carries all 5 viruses with high efficiency. *Aphis fabae* and *A. gossypii,* in addition to the previously established vectors *Macrosiphum solanifolii* and *Myzus persicae,* transmitted virus from Rembrandt tulips to *Lilium formosanum.*

CM, naturally or experimentally established in Easter lilies, was found to offer complete protection against further addition of CM or of VCM. The tulip viruses TV1 and TV2, and LT from *Lilium tigrinum,* similarly established in Easter lily, offered no protection against subsequent addition of VCM. LT in *L. tigrinum* failed to protect this species against CM, which produces killing effects in this host species.

Over 12,000 seedling Easter lilies grown from seed of diseased parents showed no evidence of seed carriage of any lily virus.

It is suggested that the 5 viruses studied be considered strains or subspecies of tulip-breaking virus, which *Marmor tulipae* Holmes may be amended to describe, and which will correspond to *Tulipa virus 1* (Cayley) Smith if the alleged hosts *Hyacinthus* and *Narcissus* are omitted.

VCM is considered a mutant from CM that appears in Easter lily stocks

carrying CM. Easter lily producers, already ignoring CM because it is impracticable to rogue in this species, are advised to ignore VCM also, for the same reason, although the latter produces some cull plants.

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BACTERIAL SOFT ROT OF SPINACH

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Bacterial soft rot of spinach is one of the most frequently observed diseases of spinach in transit and on the market. Records of the Fresh Products Inspection Division, Fruit and Vegetable Branch, United States Department of Agriculture, Chicago, Illinois, during 1936–43 show that this disease caused practically all the decay noted in spinach from all shipping regions. In many shipments there was only a trace, but in others the decay ranged as high as 65 per cent in some baskets, with an average of 40 per cent for the car.

Although many writers have assumed that the soft rot of spinach is caused by *Erwinia carotovora* (L. R. Jones) Holland, no critical studies have been made to determine the identity of the causal organism. This paper is the result of studies on the morphological, physiological, and pathological characteristics of the causal organism of the soft rot of spinach.

The first manifestations of the disease are water-soaked areas on the leaves (Fig. 1, A). These are followed by a rapid softening of the tissues which often become light yellow, wet, and mushy. High humidity and high temperature (24 to 30° C.) favor the rapid spread of the disease, which may completely destroy the leaf. If diseased leaves are removed to dry air the decay may be checked and the affected tissues become dry and brittle (Fig. 1, B).

In the present study three isolates from rotted spinach and two isolates from rotted potatoes were used. Earlier physiological and pathological tests had shown that the potato isolates were authentic *E. carotovora*.

MORPHOLOGY AND STAINING REACTIONS

Methods. Morphological characteristics of the isolates were determined from 48-hour-old cultures grown at 24° C. on beef extract nutrient agar adjusted to pH 6.9. For demonstration of form and size, "negative" preparations from smears were made with one per cent nigrosin. Fisher and Conn's (3) flagella stain was used to determine the number and position of flagella. Gram reaction was determined according to Hucker and Conn's (4) modification of Gram's stain. Ziehl-Nielsen's method was employed for acid-fast properties. The Anthony (1) method of capsule staining was employed.

Morphological characteristics. The isolates from spinach and the *E. carotovora* isolates from potato were rods, 0.4 to 0.6 by 3.6 to 4.5 microns, occurring singly, occasionally in chains; no spores; no capsules. The organ-

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isms were motile by 2 to 5 peritrichiate flagella. They were Gram-negative and not acid-fast. They stained readily with carbol fuchsin and crystal violet. In general it may be stated that the morphological characteristics of the cells of the different isolates were not sufficiently distinct to separate them one from another.

CULTURAL CHARACTERISTICS

Potato-dextrose agar (pH 7.0). On slants, growth of all isolates was thin, grayish-white, moist, glistening, butyrous. Medium was not discolored.

Potato. On steamed potato cylinders the isolates formed a smooth, slimy raised, cream-white growth.

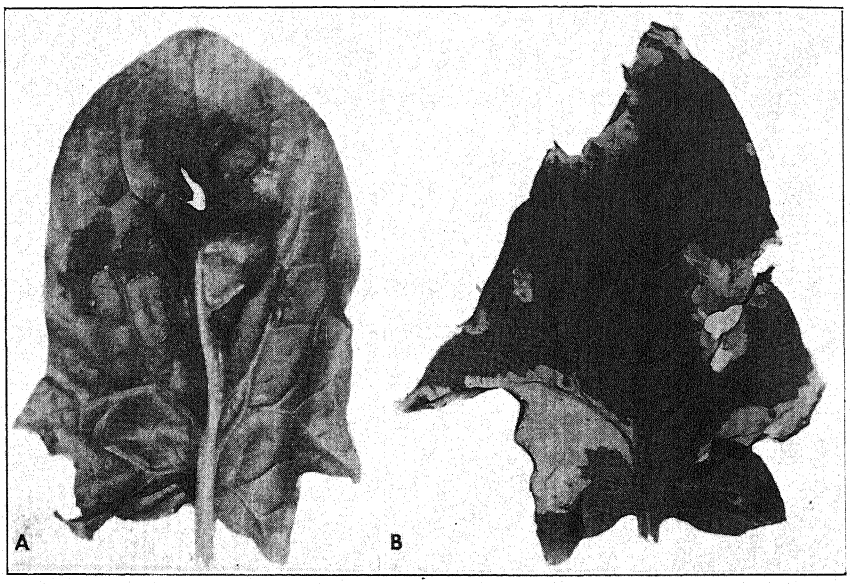


FIG. 1, A. Early stage of bacterial soft rot of spinach showing water soaked areas. B. Diseased areas which have dried and become brittle after exposure to dry air.

Beef-extract-agar streak (pH 6.9). Growth on this medium was grayish-white, glistening, butyrous, thin, margins often becoming more or less irregular.

Beef-extract agar (pH 6.9). In dilution plates after 48 hours colonies were circular, convex, grayish-white, glistening, smooth, with margins entire.

Uschinsky's solution (pH 6.8). Growth of all isolates in this medium was abundant after 24 hours.

Litmus milk (pH 7.0). All isolates produced acid in litmus milk. There was coagulation with separation of whey. No peptonization occurred.

Plain gelatin stabs. Growth became visible after 48 hours. In all cases liquefaction was at first infundibuliform but soon became stratiform. Two of the spinach isolates completely liquefied this medium after 3 weeks. The remaining isolates had completely liquefied the medium after 4 weeks.

PHYSIOLOGY

Certain of the recommendations of the Committee on Bacteriological Technique, Society of American Bacteriologists, as given in the Manual of Methods for Pure Culture Study of Bacteria (5), were closely followed in the biochemical studies. The cultures were made in duplicate and were incubated at room temperature which averaged 24° C.

BIOCHEMICAL CHARACTERISTICS

Relation to free oxygen. When grown in Smith fermentation tubes, growth occurred first in the open arms and then progressed to the domes, indicating that the organisms were facultative anaerobes.

Nitrate reduction. The alpha-naphthylamine-sulfanilic-acid test was employed on 1-, 2-, 5-, 7-, 10-, and 14-day-old cultures of the different isolates. The organism *Escherichia coli* was used as a control. Nitrates were reduced to nitrites in all of the cultures.

Indole production. The isolates in Bacto-tryptophane broth (pH 6.8) were tested by the Kovacs test for indole production at the end of 7, 14, and 21 days. No positive reaction was obtained with any of the isolates. When *Escherichia coli* was used as a control organism, a positive test for indole was obtained.

Ammonia production. Strips of filter paper saturated with a freshly prepared Nessler's solution were hung over 36- and 48-hour beef-extract broth cultures of the various isolates. No ammonia production was observed in any of them.

Hydrogen sulphide. Strips of lead acetate paper failed to turn black when hung over beef extract broth cultures of the various isolates, indicating that no hydrogen sulphide was being formed. *Escherichia coli* used as a control organism gave a positive test for hydrogen sulphide.

Hydrolysis of starch. Streak inoculations were made in beef-extract agar containing 0.2 per cent soluble starch. After five days, the surfaces of the plates were flooded with a saturated solution of iodine in 50 per cent alcohol. None showed any clear zone outside the area of growth, indicating that no diastatic action had occurred.

Hydrogen-ion relations. The spinach and the *E. carotovora* isolates all grew within a pH range of 4.2 to 10.0. Most rapid growth during 48 hours occurred from pH 6.2 to 7.5. Growth in nutrient broth was inhibited at pH 4.0 in the acid range and pH 11.0 in the alkaline range.

The results of the cultural and biochemical tests indicate that the isolates are closely related, if not identical.

CARBON METABOLISM

Sugars, alcohols, and glucosides were added to the modified synthetic medium of Ayres, Rupp and Johnson (2).

The basal medium was adjusted to pH 7.0 and sterilized by autoclaving. Brom-cresol purple at a concentration of 0.02 per cent was added as an

indicator. One per cent of sugar was added to the synthetic base and used as a liquid medium. In order to avoid breaking down during sterilization, the sugars lactose, levulose, maltose, sucrose, and raffinose were sterilized by filtration through a Berkefeld filter. Twenty-four carbon sources were utilized. All media were incubated for 6 days at 27° C. to check their sterility before using. Inoculations were made in duplicate. In all the fermentation studies color change from purple to yellow was considered the index of fermentation.

The three spinach isolates fermented arabinose, rhamnose, xylose, dextrose, levulose, maltose, galactose, mannose, raffinose, sucrose, cellobiose, glycerol, mannitol, sorbitol, dulcitol, inositol, salicin, and arbutin. The *E. carotovora* isolates fermented all of these carbohydrates except maltose, sorbitol, and dulcitol. None of the spinach nor *E. carotovora* isolates fermented melezitose, starch, dextrin, erythritol, inulin, or glycogen.

Gas production. Smith fermentation tubes were employed. One per cent of the various sugars was added to the synthetic base and used as a liquid medium. The sugars lactose, levulose, maltose, sucrose, and raffinose were sterilized by filtration through a Berkefeld filter. The remainder were sterilized by autoclaving. Gas production by the spinach and *E. carotovora* isolates was observed in arabinose, dextrose, xylose, raffinose, sucrose, mannitol, salicin, and arbutin.

The spinach and the *E. carotovora* isolates had similar reactions in the various carbohydrate media used with the exception that the latter did not ferment maltose, sorbitol, and dulcitol. These differences in carbon metabolism are not considered sufficient to differentiate the spinach and *E. carotovora* isolates.

PATHOGENICITY STUDIES

Spinach leaves were washed in tap and distilled water, after which they were divided into two lots. Uninjured leaves in one set of damp chambers were sprayed with water suspensions of the various spinach and *E. carotovora* isolates. A second lot of leaves was wounded by scratching with sterile needles. The leaves were then sprayed with water suspensions of the various isolates, after which they were left at room temperature (25° C.). Scratched and unscratched leaves sprayed with distilled water served as controls. Infection in the form of water-soaked areas around the needle scratches was first apparent 18 hours after inoculation. Numerous, small water-soaked areas were observed on the uninjured, inoculated leaves after 30 hours. All injured and uninjured inoculated leaves were completely rotted after 72 hours. Uninoculated leaves did not become infected. The organisms, readily recovered in pure culture from the infected areas, were used in further inoculation experiments.

Injured and uninjured spinach plants growing in pots in the green-house were sprayed with 24-hour-old suspensions of the various isolates. Injured and uninjured plants sprayed with distilled water served as controls. Water-soaked areas in the immediate vicinity of needle scratches were appar-

ent on some of the leaves 15 hours after inoculation. Infection of uninjured, inoculated leaves was observed 24 hours after inoculation. Control plants were free of the disease 36 hours after inoculation.

These experiments show that bacterial soft rot infection of spinach leaves may take place either through injured or uninjured tissue. Infection apparently occurs at a more rapid rate through wounds. Observations throughout the course of the experiments indicated that the spinach and the *E. carotovora* isolates were equally virulent to spinach.

In order to obtain more information on the effect of the various temperatures on the incidence of soft rot of spinach, leaves were washed in tap and distilled water. One lot was atomized with a water suspension of one of the spinach isolates, one lot with a suspension of *E. carotovora,* and a third lot with distilled water. The various lots were placed in damp chambers which were, in turn, placed in refrigerators where temperatures of 4.5°, 7.5° and 10.0° C. were constantly maintained. At the end of 5 days no rot had developed in the lots held at 4.5° and 7.5° C. During this period one leaf in the damp chamber held at 10.0° C. became infected. After 8 days the inoculated spinach held at 4.5° was free of infection, one leaf in the damp chamber held at 7.5° was infected, while all the leaves in the damp chamber held at 10° were rotted. Control leaves remained uninfected.

These data indicate that during a 5 to 8 days' transit period the development of spinach soft rot would be very materially checked if a temperature of 4.5° C. was maintained. This is borne out by the Fresh Products Inspection Division records, which show that the incidence of spinach soft rot is often held to one per cent or less if the temperature in transit does not exceed 4.5° C.

Further inoculation tests on potato tubers with the spinach and the *E. carotovora* isolates showed that all were pathogenic to this host. It was noted that the rot induced by the *E. carotovora* isolates occurred at a more rapid rate and was more extensive than that caused by the spinach isolates.

SUMMARY

Bacterial soft rot of harvested spinach is described. Morphological characteristics of the cells of spinach soft rot isolates and of *Erwinia carotovora* cultures were not sufficiently distinct to separate them one from another. Results of cultural and biochemical tests indicate that the spinach and the *E. carotovora* isolates are closely related if not identical. Differences in carbon metabolism are not considered sufficient to differentiate the spinach and the *E. carotovora* isolates. Pathogenicity experiments show that bacterial soft rot of spinach may take place through injured or uninjured leaves. Isolates from rotted spinach proved pathogenic to potato tubers. Spinach soft rot may be controlled in transportation and marketing if a temperature of 4.5° C. is maintained for 8 days.

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EXPERIMENTS WITH PEA SEED TREATMENTS IN COLORADO[1]

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INTRODUCTION

Increasing difficulty has been encountered in obtaining satisfactory stands in some of the older pea-growing sections of Colorado. Although the effects of pea seed treatments have been reported from other states (4, 5, 6, 8, 9, 11, 12), conditions under which peas are grown in Colorado differ from those of most other pea-growing sections. In fact, the pea-growing regions within the state are extremely variable as to soils, altitude, and climate. Colorado soils vary from adobe and adobe-like aggregates through sand and sandy-loam to gravel. Altitudes of the pea-growing sections vary from 4,200 feet at Rocky Ford to 7,600 feet in the San Luis Valley. Lower temperatures are associated with the higher altitudes. Cultural practices also vary somewhat, subirrigation being used in the San Luis Valley and row irrigation being practiced in the rest of the state. Since it was not known whether seed treatment materials used in other states would have beneficial effects in Colorado, a series of experiments was designed to determine the effect of fungicidal seed treatments on the emergence and growth of peas under Colorado conditions.

EXPERIMENTAL METHODS

During the summer of 1943, twelve experimental plots of peas were planted in the principal pea-growing areas of Colorado: the northern section, the San Luis Valley, and the Arkansas Valley. Four seed treatments and an untreated control were used in all tests. The chemicals used and the rates of application were New Improved Ceresan (5 per cent ethyl mercury phosphate) at 1 ounce per bushel of seed, and Spergon (98 per cent tetrachloroparabenzoquinone), Arasan (50 per cent tetramethylthiuramdisulfide), and yellow Cuprocide (93 per cent yellow cuprous oxide), each at 2 ounces per bushel.

One plot of each of the four canning varieties, Alaska, Perfection, Wisconsin Sweet, and Green Admiral was planted in the Loveland-Berthoud district. Variety Rogers' 95 was used in three plots in the San Luis Valley,

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one plot at Rocky Ford, and one at Fort Collins. Variety Little Marvel was used in three plots in commercial market gardens near Fort Collins.

The plot at Rocky Ford, one plot in the San Luis Valley, and one at Fort Collins were planted by hand, but all others were planted with a garden drill. All plots were designed as either Latin squares or randomized blocks, and data obtained were analyzed by the analysis of variance method.

In the Little Marvel tests, 50 pounds of seed were divided into 5 lots of 10 pounds each. Four of these lots were treated and the fifth lot was left untreated. Planting was done with a tractor-drawn pea drill. Stand counts were made by marking off sample strips at each end and in the center of the field.

Stand counts were made for all tests. Yield records were obtained from five of the tests. Living and dead plants were counted on two of the plots at picking time and the percentage of living plants determined. To analyze these results the percentages were converted into degrees of an angle ($\sin^2 \Theta$) by use of Bliss' tables (1, 2).

EFFECTS ON EMERGENCE

Northern Colorado.—No significant stand increases were obtained from any of the seed treatments in the Berthoud district (Table 1). Spergon treatment resulted in a highly significant[6] stand increase over that from untreated or Cuprocide-treated seed of variety Wisconsin Sweet in a test near Loveland. In this same test stands from New Improved Ceresan and Arasan treatments were significantly[6] greater than those from untreated or Cuprocide-treated seed. In the variety Green Admiral, near Loveland, all treatments resulted in highly significant increases in stand. The stand from Spergon-treated seed was significantly better than that from any other treatment.

In plot 5 in the Fort Collins area Little Marvel seed treated with New Improved Ceresan or Arasan gave stands which were significantly better at the 1 per cent level than stands from untreated seed. Cuprocide was better at the 5 per cent level. The stand from New Improved Ceresan was significantly better than that from Spergon or Cuprocide. In a second planting of this variety (Plot 6) stand increases which were highly significant resulted from all treatments. New Improved Ceresan, Spergon, and Arasan produced stands which were significantly better at the 1 per cent level than Cuprocide. In a third planting (Plot 7) Spergon and Cuprocide gave significant stand increases over the untreated.

In the plot of Rogers' 95 at Fort Collins, Spergon, Arasan, and Cuprocide treatments all gave stand increases which were highly significant. New Improved Ceresan was significantly better than the untreated. The stand from Spergon treatment was better than the stand from New Improved Ceresan at the 5 per cent level of significance.

[6] "Highly significant" as used in this paper refers to the 1 per cent level of significance; "significant" refers to the 5 per cent level of significance.

TABLE 1.—*Effect of seed treatment on emergence*

Plot No.	Variety	Location	Mean numbers of plants emerged per plot					Diff. req. for sig. 5% level	Diff. req. for sig. 1% level
			Untreated	Spergon	Arasan	Cuprocide	New Improved Ceresan		
1	Alaska	Berthoud	649.6	675.4	641.2	604.6	626.4	N.S.[c]	N.S.
2	Perfection	Berthoud	976.6	1016.0	829.8	738.0	931.8	N.S.	N.S.
3	Wisconsin Sweet	Loveland	580.6	689.0	657.8	555.6	677.2	74.3	104.1
4	Green Admiral	Loveland	351.8	528.6	476.2	487.2[b]	483.4	41.0	57.5
5	Little Marvel Planted April 16	Ft. Collins	189.3	207.6	227.0	217.3[b]	244.3	25.3	36.8
6	Little Marvel Planted May 1	Ft. Collins	460.6	513.6	507.6	486.0[b]	516.3	12.0	17.5
7	Little Marvel Planted June 8	Ft. Collins	333.6	407.0	364.3	394.6[b]	370.0	56.0	81.5
8	Rogers' 95[a]	Ft. Collins	144.8	190.8	186.2	182.2	166.6	20.2	27.9
9	Rogers' 95[a]	Rocky Ford	34.6	76.8	79.0	82.0	88.8	10.7	15.0
10	Rogers' 95	Center	98.0	128.8	121.8	101.2	162.0	22.6	31.6
11	Rogers' 95[a]	Center	130.6	183.4	182.4	196.8	184.6	14.1	19.7
12	Rogers' 95	Sanford	16.0	61.2	26.0	47.4	49.6	22.7	31.8

[a] Planted by hand. All others were drilled.
[b] Graphite mixed with Cuprocide.
[c] N.S. = not significant.

Arkansas Valley.—At Rocky Ford all seed treatments resulted in stand increases which were highly significant. The increase from New Improved Ceresan was significantly better than the increase from Spergon, but not significantly better than increases from Arasan or Cuprocide.

San Luis Valley.—Highly significant stand increases were obtained from all treatments in the hand-planted plot 11 on the San Luis Valley Demonstration Farm near Center. In the drilled plot 10, New Improved Ceresan was better than any other treatment at the 1 per cent level. Spergon and Arasan were significantly better than no treatment.

Although all stands were poor on the Sanford plots, Spergon and New Improved Ceresan produced stands that were better at the 1 per cent level and Cuprocide produced stands that were better at the 5 per cent level than those of the untreated seed. Spergon was superior to Arasan at the 1 per cent level.

EFFECTS ON YIELD AND SURVIVAL OF PLANTS

No significant differences in yield were obtained in the Alaska variety at Berthoud (Table 2). In Green Admiral the increase from Spergon over all other treatments was highly significant.

The yields of Little Marvel represent the total yield of pod peas obtained from the 10 pounds of seed for each treatment. This was not a replicated plot so data could not be analyzed statistically. However, the field selected for this test was uniform so it seems unlikely that the results were due to chance. Increases over yields from untreated seed were: New Improved Ceresan 22.2 per cent, Cuprocide 18.4 per cent, Arasan 17.6 per cent, Spergon 16.6 per cent.

Yield differences were not significant in the San Luis Valley or in the Rogers' 95 trial at Fort Collins. However, increases in yield from New Improved Ceresan-treated seed approached significance in both plots on the Demonstration Farm at Center.

Percentages of living plants at picking time, based on original stand, are shown in table 3. Differences were not significant in either plot.

DISCUSSION

There are two phases to consider in dealing with root-rotting fungi. The first is the rotting of the seed or the destruction of the young seedling before it emerges from the ground. The second phase is the attack made by the parasites on the older plants. These tests show that seed treatment will generally control the first phase. That this has an indirect effect on the second phase is shown by the counts of living plants at harvest time. These counts show that the same percentage of plants live to reach maturity, regardless of seed treatment. This indicates that seed treatment is effective only during the early life of the plants. However, a better stand obtained by treating the seed will result in a greater number of plants reaching maturity.

TABLE 2.—*Effect of seed treatment on yield*

Plot No.	Variety	Location	Mean yield[a] per plot					Diff. req. for sig. 5% level	Diff. req. for sig. 1% level
			Untreated	Spergon	Arasan	Cuprocide	New Improved Ceresan		
1	Alaska	Berthoud	28.2	29.4	28.6	29.4	27.2	N.S.	N.S.
4	Green Admiral	Loveland	24.3	33.7	23.3	26.0	22.0	2.1	2.9
10	Rogers' 95	Center	229.2	219.0	249.2	233.2	335.6	110.9	155.5
11	Rogers' 95	Center	444.2	482.8	420.2	506.6	523.4	81.0	113.6
8	Rogers' 95	Ft. Collins	121.6	132.8	135.6	141.4	117.6	29.8	41.1
5	Little Marvel	Ft. Collins	607.0	708.0	714.0	719.0	742.0	.....	.....

[a] Yields in tests 1 and 4 are in ounces of shelled peas; in tests 10, 11, and 8, in ounces of pod peas; and in test 5, in total pounds of pod peas.

TABLE 3.—*Effect of seed treatment on survival of plants*

Plot No.	Variety	Location	Per cent of living plants at picking time[a]					
				Untreated	Spergon	Arasan	Cuprocide	New Improved Ceresan
8	Rogers' 95	Ft. Collins	Actual per cent	60.64	66.46	56.24	66.30	50.90
			Converted value	51.43	54.71	48.68	54.62	45.52
11	Rogers' 95	Center	Actual per cent	66.98	59.34	59.82	64.64	63.40
			Converted value	52.87	50.53	50.75	53.64	52.87

[a] Differences are not significant.

In these experiments the same seed treatment did not give similar results under all conditions. This has also been observed by workers in other states (7, 8, 10, 11, 13). The inconsistency may be attributed to a number of factors. Jones (7) showed that variations in soil moisture near the time of planting and differences in soil temperatures cause wide variations in the results obtained from seed treatment. McNew (10) points out that different seed lots vary greatly in their ability to withstand seed decay. Experiments at the Colorado Agricultural Experiment Station indicated that seed treatments vary in their effectiveness against different fungi (3).

There is a great deal of variation in Colorado soils. The soils in which the tests were conducted varied as to type, fertility, moisture content at planting time, and fungus infestation. Isolations from diseased plants showed that several pathogenic fungi were present in some of the soils. While no attempt was made to determine the relative distribution of these fungi, *Fusarium solani* (Mart.) v. *mar. ii* (App. et Wr.) Wr. f. 2 Sny. was obtained most frequently in the isolations. Other pathogens isolated were *Ascochyta pinodella* Jones, *Pythium* sp., and *Corticium vagum* B. et C. var. *solani* Burt. (*Rhizoctonia solani* Kuhn.).

Spergon gave the greatest increase in stand in seven of the tests, New Improved Ceresan was first four times, and Yellow Cuprocide led in one trial. In no case was the untreated lot superior. From tests in New York State McNew (8) concluded that New Improved Ceresan should not be used on peas because it was too injurious when applied at 1 ounce per bushel of seed. Our trials in Colorado do not substantiate this, since New Improved Ceresan gave the best results in some trials.

In the trial with greatest increase in stand from Cuprocide the seed was planted by hand. During planting of the other plots it was noted that Cuprocide-treated seed tended to bind in the drill, so that many of the seeds were cracked. Addition of graphite seemed to eliminate this trouble. This may explain why the stand counts were low in some of the Cuprocide-treated plots when graphite was not used.

While these results are by no means conclusive as to the superiority of one treatment over another, they do show that in most cases any of the materials tested gave better stands than no treatment. Work to determine more of the factors which influence the effectiveness of seed treatments is necessary before complete recommendations can be made for any specific locality.

SUMMARY

Pea seed treatments with Spergon, New Improved Ceresan, Arasan, and Yellow Cuprocide in three pea-growing sections of Colorado generally resulted in increased stands. Although Spergon and New Improved Ceresan gave the best results in the majority of the tests, increases in stand and yield varied in the different trials, so that no one treatment was consistently outstanding.

COLORADO AGRICULTURAL EXPERIMENT STATION,
FORT COLLINS, COLORADO.

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9. ———. Relative effectiveness of organic and inorganic fungicides as seed protectants. (Abstr.) Phytopath. 33: 9. 1943.
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13. WALKER, J. C., E. J. DELWICHE, and PAUL G. SMITH. Pea seed treatment with chemical dusts. Jour. Agr. Res. [U.S.] 61: 143–147. 1940.

PHYTOPATHOLOGICAL NOTES

Effect of Penicillin on a Plant Pathogen.—Penicillin, whose bacteriostatic properties were first recognized by Fleming,[1] has of late excited considerable interest among pathologists dealing with bacterial diseases of the human body. The product has been described as particularly active against numerous bacterial animal pathogens that are gram-positive. No reference to experiments with plant pathogens has been found by the authors of this note. Because we have been particularly interested in a gram-positive bacterial plant pathogen that is extensively distributed in this region and since we have sought to try anything that promises a degree of control, penicillin was included in the program of experiments.

The first problem in connection with the use of penicillin was that of procuring the substance. Cultures of the penicillin-producing fungus, *Penicillium notatum,* were obtained and grown in sterile, large but shallow, glass vessels. Later a modification of the apparatus described by Clifton,[2] very much like that used in the manufacture of vinegar, was set up. So much of the penicillin-containing liquid was thus made that a considerable quantity that would otherwise have been wasted has been made available for medical use. Media used were a modified Czapek-Dox[3] and corn-grain decoction made by boiling approximately 50 grams of the dry grains in 1 liter of water.

The plant pathogen subjected to the action of penicillin was the gram-positive bacterium, *Erwinia carnegieana* Standring,[4] that has caused extensive destruction of the giant cactus in Arizona and Mexico. Petri-dish cultures were seeded by growing the organism in broth, pouring the latter on sterile agar plates, and draining off the excess broth suspension. Two hours later a small glass penicillin cylinder, approximately 9.5 mm. in length and 5 mm. in diameter, was seated in the center of the Petri-dish culture, following the procedure described by Abraham *et al.*[3] For comparison a similar series of plates was set up for *Staphylococcus aureus,* the bacterium that is used as a test in experiments with the penicillin drug.

Penicillin suppresses the cactus plant pathogen as it does *Staphylococcus aureus* (Fig. 1). The clear zone around the penicillin chamber in the culture of *Staph. aureus* is even smaller than that in the culture of the plant pathogen. However, the cultures were grown at room temperature, which is more favorable for the development of the plant pathogen.

Besides the results with *Erwinia carnegieana,* we have indications that the gram-positive *Corynebacterium sepedonicum* likewise is susceptible to

[1] Fleming, Alexander. On antibacterial action of cultures of *Penicillium,* with special reference to their use in isolation of *B. influenzae.* British Jour. Exp. Path. 10: 226–236. 1929.

[2] Clifton, C. E. Large-scale production of penicillin. Science 98: 67–70. 1943.

[3] Abraham, D. L., A. D. Gardner, E. Chain, N. G. Heatley, C. M. Fletcher, M. A. Jennings, and H. W. Florey. Further observations on penicillin. Lancet 241: 177–188. 1941.

[4] Lightle, Paul C., Elizabeth T. Standring, and J. G. Brown. A bacterial necrosis of the giant cactus. Phytopath. 32: 303–313. 1942.

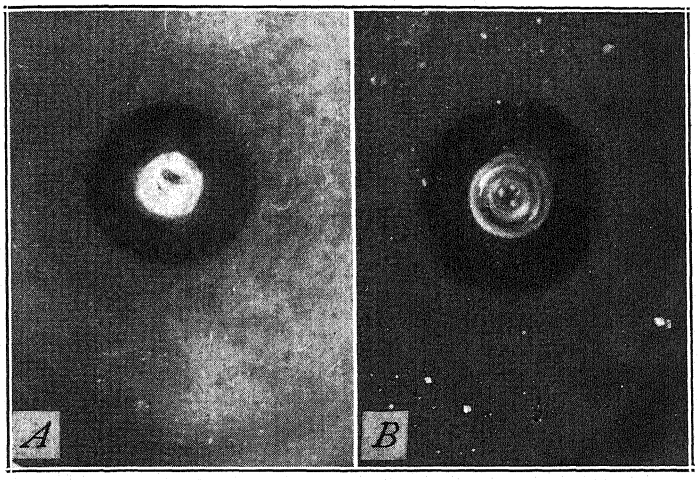


FIG. 1. Effect of penicillin on (A) *Staphylococcus aureus* and (B) *Erwinia carnegieana*. The penicillin-containing liquid was placed in the small glass cylinders after the plates were seeded. Diffusion of the penicillin into the agar suppressed bacterial growth in the circular "clear zones."

the action of penicillin. The bacterial-ring-rot pathogen is slower growing and somewhat more difficult to work with on that account.—J. G. BROWN and ALICE M. BOYLE, University of Arizona, Tucson, Ariz.

Improved Cork-borer Method for Inoculating Trees.—The writer has used the previously described cork-borer method for inoculating trees[1] and has found it satisfactory when inoculating small numbers of trees. During the summer of 1943 approximately 1800 chestnut hybrids were inoculated at from one to three points on each tree, resulting in approximately 3300 inoculations. In large-scale inoculation work the elimination of even one movement means a considerable saving in time; therefore, the writer devised an improved instrument. When using the ordinary cork borer it is necessary to pick up the accessory wire or rod plunger to punch out the cork plug. When using the improved instrument the thumb of the hand that holds the instrument presses against the plunger, which is an integral part of the borer. The movement, otherwise made to reach for a detached plunger, is now used to pick up the inoculating needle or other item, or is eliminated.

Figure 1, A, shows the improved instrument assembled; figure 1, B, shows the instrument disassembled. To construct the improved device, first force off the cross bar of the cork borer; heat the upper end of the tube to redness and hammer the end lightly to form an inner lip or shoulder. Replace the cross bar on the tube at a point about 1½ inches from the upper end of the tube; braze or solder securely to the tube.

The plunger may be made from copper, aluminum, or plastic rod, of slightly less diameter than the inner bore of the tube. The upper end of the

[1] Wright, Ernest. A cork-borer method for inoculating trees. Phytopath. 23: 487–488. 1933.

plunger for a distance of about $\frac{5}{8}$ inch should be of a smaller diameter to allow passage through the flanged opening in the upper end of the tube. The writer found that an inoculating needle holder of the Rosenberger and Greenman type could be adapted as a plunger with a minimum of effort for a $\frac{1}{4}$ inch cork borer. The aluminum rod of this holder is cut off at about $\frac{5}{8}$ inch from the plastic handle; the end of the rod is bored and tapped to take a machine screw, which holds the coiled spring in place.

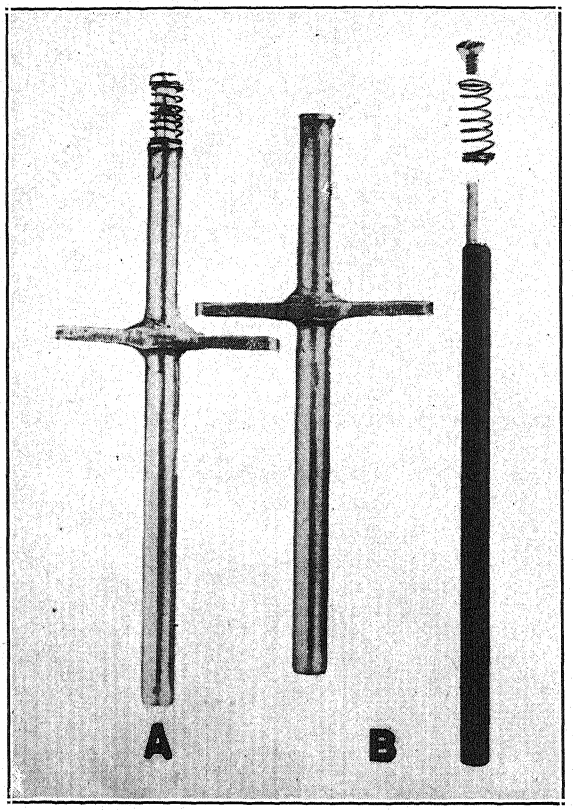


FIG. 1. A. Modified cork borer assembled. B. Modified cork borer disassembled, showing at right the plunger with spring and machine screw detached.

Some pathologists prefer not only to make uniform incisions when inoculating, but also to use uniform amounts of inoculum. A cork borer that is one or two sizes smaller than the borer that makes the incision can be used to cut plugs of inoculum in a Petri dish. The plugs may be cut in the laboratory so that they will be ready to be lifted out with the inoculating needle. The inoculum should be stiff for best results.—RUSSELL B. CLAPPER, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Lime in the Post-Arsenical Sprays as a Means of Reducing Arsenical Injury to Peaches.[1]—Several workers have investigated the effects of calcium hydroxide, calcium carbonate, and mixtures of these two compounds on the formation of soluble arsenic from lead arsenate in a water solvent. It has been shown that calcium hydroxide is very effective in reducing the concentration of water-soluble arsenic, that calcium carbonate increases the formation of soluble arsenic compared with that of lead arsenate alone,[2] and that combinations of calcium hydroxide and calcium carbonate are effective in

TABLE 1.—*Effect of a delayed lime application on the development of arsenical injury to peach foliage*

Treatment no.	Materials[a] used at shuck split and first cover applications	Materials[a] used at second cover applications	Foliage injury			
			Golden Jubilee variety[b]		Fireglow variety	
			Defoliation	Remaining leaves with necrotic areas from spray injury	Defoliation	Remaining leaves with necrotic areas from spray injury
			Per cent	*Per cent*	*Per cent*	*Per cent*
1A	Acid lead arsenate 2 lbs., sulphur 8 lbs., per 100 gals.	Sulphur 8 lbs., lime 8 lbs.	15.0	35.0	5.0	10.0
1B	do	Sulphur 8 lbs.	75.0	100.0	30.0	90.0
2A	Acid lead arsenate 2 lbs., hydrated lime 6 lbs., sulphur 8 lbs.	Sulphur 8 lbs., lime 8 lbs.	3.0	7.0	1.0	5.0
2B	do	Sulphur 8 lbs.	27.0	71.0	15.6	67.5
3A	Acid lead arsenate 2 lbs., hydrated lime 16 lbs., sulphur 8 lbs.	Sulphur 8 lbs., lime 8 lbs.	0.0	Trace	Trace	Trace
3B	do	Sulphur 8 lbs.	0.0	0.0	Trace	3.3

[a] Magnetic Brand Sulphur (325-mesh) used in all cases.
[b] The foliage on the Golden Jubilee trees in the 1B and 2B treated blocks was lighter green than trees in the corresponding A blocks.

reducing the formation of soluble arsenic from lead arsenate.[3] This last statement is made because Van der Meulen and Van Leeuwen report that ". . . carbonation takes place very slowly, and . . . as long as there is any unchanged calcium hydroxide the amount of soluble arsenic is very low."

Arsenical injury to peach foliage and twigs in New Jersey often has been observed to appear a rather long time (as much as one month) after the application of the last arsenical spray. It seemed probable that this delayed

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Plant Pathology.
[2] Ginsburg, Joseph M. Chemical effect on lead arsenate of certain salts which may be present in soil and spray waters. Jour. Agr. Res. [U.S.] 60: 199–205. 1940.
[3] Van der Meulen, P. A., and E. R. Van Leeuwen. A study of lead arsenate and lime spray mixtures. Jour. Agr. Res. [U.S.] 35: 313–321. 1927.

arsenical injury was due either to the carbonation of the lime present or to the removal of the calcium hydroxide by weathering. To test the value of adding lime to the post-arsenical sprays as a means of preventing the occurrence of late-developing injury, three tests were made during the growing season of 1943.

In tests 1 and 2, peach trees were sprayed with mixtures containing sulphur (8 lbs.), acid lead arsenate (2 lbs.), and various concentrations of high-calcium hydrated lime (0, 6, and 16 lbs. per 100 gals.) at two consecutive spray periods. Sprays were applied at the usual times, those of the shuck split and first cover applications. In the third test, one application of lead arsenate, sulphur, and lime was made about 6 weeks after shuck split.

In all tests, each group of similarly treated trees was divided, after application of the sprays containing lead arsenate, into two lots, one lot receiving a sulphur-hydrated lime spray, and the other sulphur alone. This application of sulphur and lime or sulphur alone followed the last lead arsenate spray by 12 to 19 days.

Test 1 was in southern New Jersey on J. H. Hale and Elberta peach trees. Environmental conditions were not conducive to arsenical injury, and consequently no advantage was noted from the use of the delayed lime application. Arsenical injury was not pronounced in any treatment, and the amount of injury did not appear to increase in any block after the delayed lime application.

Test 2 was in a block of Golden Jubilee trees in New Brunswick where environmental conditions, during the test, were conducive to the development of arsenical injury. The data (Table 1) were taken two weeks after the last spray was applied.

Test 3 was in New Brunswick on trees of the Fireglow variety. When the delayed lime spray was applied, foliage injury was already visible. The data (Table 1) were obtained by making foliage counts 18 days after the last spray was applied.

From the results of these experiments, it is apparent that the inclusion of hydrated lime in a spray following by approximately two weeks one containing lead arsenate may materially reduce the amount of foliage injury to peaches that results from the use of lead arsenate.—ROBERT H. DAINES, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

A Method of Inoculating Peach Seedlings with Crown Gall without Using Punctures.—The host range of crown gall has largely been determined either by making artificial injuries and inserting the crown-gall organism from pure cultures, or by growing the host in infected soil. The results presented in this paper show a water suspension from uninjured galls to be an efficient means of placing the crown-gall organism in the soil in contact with peach seedlings that were not artificially injured. Banfield[1] has shown that unin-

[1] Banfield, W. M. Life history of the crown-gall organism in relation to its pathogenesis on the red raspberry. Jour. Agr. Res. [U.S.] 48: 761–787. 1939.

jured crown gall on raspberries can liberate in water large numbers of the crown-gall organisms. Smith and Cochran[2] have reported that a high percentage of crown gall occurs on stone fruits in nurseries on land having no previous history of crown gall, and have associated this trouble with irrigation water that was contaminated with the crown-gall organism.

To test this avenue of infection, peach pits were planted in a dark-colored mountain potting soil (pH 8.36) that had previously been treated with chloropicrin gas. Twenty-five peach seeds that were just beginning to germinate, but were not sufficiently advanced to be injured in handling, were planted in each 5-gallon container. One week after planting, at time of emergence, the soil in some of the containers was watered with a suspension obtained from soaking 20 good-sized crown galls in 20 quarts of water over night. One quart of suspension was applied per can.

The seedlings were dug and results taken in January, after one season's growth. The results can be summarized as follows:

329 trees treated with suspensions—175, or 53 per cent, with galls
93 trees not treated (controls)— 9, or 9.6 per cent, with galls

There were some galls in the nontreated or control trees. These galls cannot be satisfactorily accounted for, but may have resulted from contamination by spattering from the infected soil of inoculated cans, or from the incomplete sterilization of the planting soil, or from the pits stratified in sand and peat. The irrigation water for these trees was from the deep wells of the Riverside city water system. It is well established that injuries are necessary for the entrance of the crown-gall organism into the host. Siegler and Bowman[3] have observed injuries made in germinating peach seedlings near where cotyledons are attached. Other injuries may occur in rapid-growing seedlings where lateral roots arise or where soil insects injure the roots. The soil in these experiments was treated with chloropicrin before being used, and was apparently free of injurious insects. This, however, does not preclude the possibility of their having been introduced with the rinse water from the unsterilized galls used in the experiment.—CLAYTON O. SMITH, University of California Citrus Experiment Station, Riverside, California.

The Term Viruliferous.—The medical term *viruliferous* was derived from *virulentus,* meaning virulent or full of virus, and *fero,* meaning bear or carry, with the idea of motion predominating. This meaning, virus-bearing, was indicated by Carsner and Stahl[1] when they substituted the established medical term *viruliferous* for the term *viriferous,* which they[2] had a little earlier coined. This latter term was so defined as to describe

[2] Smith, C. O., and L. C. Cochran. U. S. Dept. Agr. Bur. Plant Indus., Plant Dis. Reptr. 28: 160–162. 1944.

[3] Siegler, E. A., and J. J. Bowman. Crown gall of peach in the nursery. Phytopath. 30: 417–426. 1940.

[1] Carsner, E., and C. F. Stahl. Studies on curly-top disease of the sugar beet. Jour. Agr. Res. [U.S.] 28: 297–320. 1924.

[2] Stahl, C. F., and E. Carsner. A discussion of *Eutettix tenella* Baker as a carrier of curly-top of sugar beets. Jour. Econ. Ent. 16: 476–479. 1923.

the status or condition of an insect vector, in this case the beet leafhopper, *Eutettix tenellus* (Baker), in which it carries a virus, in this case the curly-top virus. Leach[3] defines *viruliferous:* "Capable of inducing a disease by feeding on the suscept."

The term *viruliferous* has gradually come into extensive and effective use in plant pathology in the above sense. This precise usage may be corrupted unless divergent practices are avoided. Valleau[4,5] has referred to plant tissue as *viruliferous.* It would follow from this that any plant affected with a virus disease is *viruliferous.* Indeed, Johnson and Jones[6] have adopted such usage. Such corrupting of a precise, highly useful term ought to be discouraged. The Committee on Terminology (Nomenclature) of Immunology and Use of Technical Words of the American Phytopathological Society could help in this situation by recommending an appropriate, restrictive definition.

Frampton, Linn, and Hansing[7] reintroduced the term *viriferous* which they preferred to *viruliferous.* It is to be hoped that few if any other writers will follow their lead in this.—EUBANKS CARSNER, U. S. Department of Agriculture, Riverside, California.

Resistance of Guayule to the Root-knot Nematode.—At the time the emergency rubber project was started by the United States Department of Agriculture, little was known concerning the diseases of guayule, *Parthenium argentatum* Gray. Until this xerophytic plant was removed from its natural habitat and placed under cultivation, the only parasites recorded[1] were a rust, *Puccinia parthenii* (Speg.) Arthur, and dodder, *Cuscuta* sp. Recently, various workers[2, 3, 4, 5, 6, 7, 8] have given attention to certain root diseases in nurseries and experimental plantings. Among the root diseases

[3] Leach, J. G. Insect Transmission of Plant Disease. 615 pp. New York. 1940.

[4] Valleau, W. D. Experimental production of symptoms in so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopath. 31: 522–533. 1941.

[5] Valleau, W. D. Control of the common mosaic disease of tobacco by breeding. Phytopath. 32: 1022–1025. 1942.

[6] Johnson, F., and L. K. Jones. A report on a study of virus transmission by fungi and nodule bacteria of peas. U. S. Dept. Agr., Plant Disease Reporter 27: 656–658. 1943.

[7] Frampton, V. L., M. B. Linn, and E. D. Hansing. The spread of virus diseases of the yellows type under field conditions. Phytopath. 32: 799–808. 1942.

[1] Lloyd, Francis Ernest. Guayule (*Parthenium argentatum* Gray), a rubber-plant of the Chihuahuan Desert. Carnegie Inst. Wash. Publ. 139. 1911.

[2] Campbell, W. W., L. D. Leach, J. T. Presley, and W. C. Snyder. Some diseases of guayule in California. U. S. Dept. Agr. Plant Dis. Rptr. 27: 63–66. 1943.

[3] Ezekiel, Walter N. Crown rot and root rot of guayule. U. S. Dept. Agr. Plant Dis. Rptr. 27: 2–8. 1943.

[4] Hoyman, Wm. G. Preliminary evidence suggests guayule may be resistant to the root-knot nematode. U. S. Dept. Agr. Plant Dis. Rptr. 26: 476. 1942.

[5] Presley, John T. Some diseases affecting cultivated guayule in the Southwest during 1942. U. S. Dept. Agr. Plant Dis. Rptr. 27: 94–96. 1943.

[6] Streets, R. B. The susceptibility of guayule to Phymatotrichum root rot. U. S. Dept. Agr. Plant Dis. Rptr. 27: 66–68. 1943.

[7] Taubenhaus, J. J., and Walter N. Ezekiel. A rating of plants with reference to their relative resistance or susceptibility to Phymatotrichum root rot. Texas Agr. Exp. Sta. Bull. 527. 1936.

[8] Watkins, G. M. Diseases of guayule observed in Texas. U. S. Dept. Agr. Plant Dis. Rptr. 27: 591. 1943.

mentioned, it is interesting to note that guayule is not regarded as being very susceptible to *Phymatotrichum omnivorum* (Shear) Duggar, a fungus indigenous to the Southwest where guayule is being planted. Taubenhaus and Ezekiel[7] list guayule as being moderately susceptible while Streets[6] has reported only a trace of infection in the first year's growth under irrigation. Presley[5] was unable to produce infection under laboratory or field conditions.

At some locations where guayule is being planted it is quite probable that the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, occurs. Campbell, Leach, Presley and Snyder[2] have observed that plants in the older nursery beds at Salinas, California, showed light infestations. In an experiment in 1942 at Tucson, Arizona, 13 transplants[9] remained free from infestation after growing in the field in infested soil from March 20 until October. Six other transplants were grown six months in pots containing naturally infested soil. When the soil was removed from their roots, only a very slight infestation was found on one plant. The roots and tops of these six plants were pruned and returned to the original pots, and through the winter months the plants were placed in the greenhouse where the temperature was never below 20° C. Tomatoes were seeded with the six guayule plants to serve as nematode indicators. When the tomato seedlings were removed, the roots were severely infested with the root-knot nematode[10] indicating that the soil was heavily infested. Twelve months after the guayule had been returned to the pots, the roots were examined for infestation but none was apparent. The soil was returned to the pots and tomatoes were again seeded. The amount of infestation on the seedling roots indicated *Heterodera marioni* was still present in sufficient numbers to cause severe infestation of this indicator plant.

Additional evidence as to the resistance of guayule was obtained from 10 transplants growing in pots containing artificially infested, sandy soil. This experiment was started in October, 1942, in the greenhouse where the temperature was 20° C. or higher. Tomatoes were seeded in October in the pots containing the guayule transplants and when removed as seedlings the roots were severely infested with the root-knot nematode. In October, 1943, the guayule roots were removed from the pots and only a very slight infestation was observed on one of the 10 plants. The soil was returned to the pots and tomatoes were again seeded. The severe infestation observed on the seedling roots was evidence that the nematode population was still very great. These data indicate that guayule is very resistant to the root-knot nematode.—WM. G. HOYMAN, Department of Plant Pathology, University of Arizona, Tucson, Arizona.

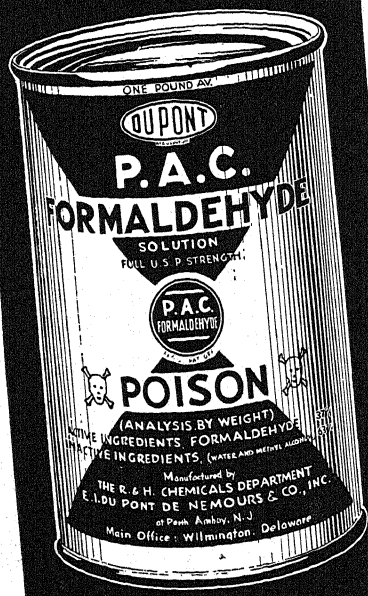
[9] The guayule transplants used in this investigation were obtained from one of the Forest Service Nurseries, Salinas, California.

[10] The root-knot nematodes occurring in the infested tomato roots were identified by Gerald Thorne, Associate Nematologist, United States Department of Agriculture, Salt Lake City, Utah.

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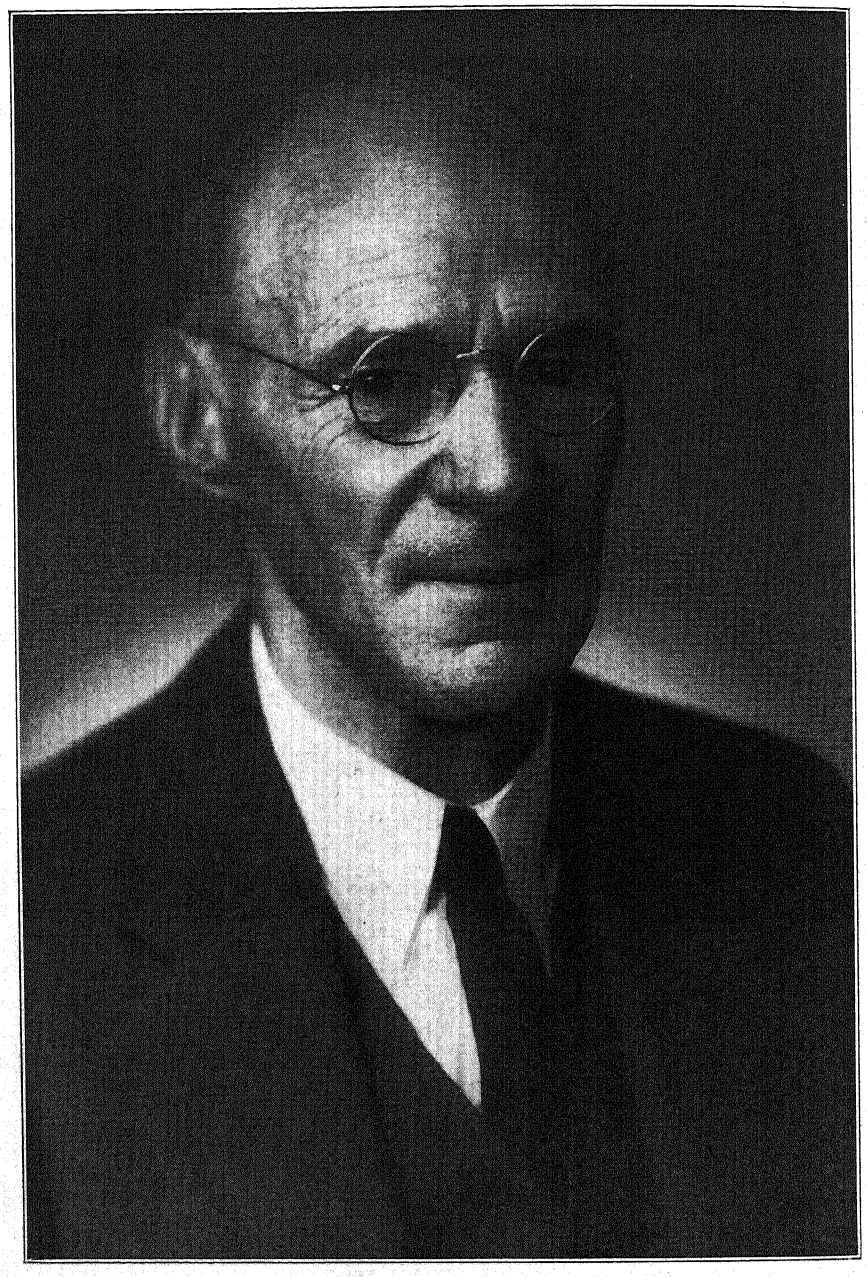
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DEANE B. SWINGLE
1879–1944

DEANE B. SWINGLE

1879–1944

H. E. Morris and F. B. Cotner

In the death of Deane B. Swingle at Bozeman, Montana, January 18, 1944, we have lost a distinguished botanist, an eminent teacher, and an excellent administrator.

Deane B. Swingle was born in Wayne County, Pennsylvania, January 6, 1879. He attended country school, was tutored by his mother, and then entered Kansas Agricultural College, obtaining his B.S. degree in 1900. He carried on graduate work at the University of Wisconsin, receiving the M.S. degree in 1901 and the Ph.D. degree in 1931.

From 1901 to 1906 he was connected with the United States Department of Agriculture, working under the direction of Erwin F. Smith.

In August, 1906, he came to Montana State College and organized the work in botany and bacteriology in the Department of Biology. A Department of Botany and Bacteriology was created in 1911, and he remained as head of this department throughout his life. He had charge of the work in botany and bacteriology, specializing in plant pathology, in the Montana Agricultural Experiment Station from 1906 to 1925. In 1931 he was made Dean of the Division of Science of Montana State College. While in this capacity he also served as acting president in President Alfred Atkinson's absence. When A. L. Strand became President, Dr. Swingle was formally named as vice-president and continued to act in this capacity until his death.

Dr. Swingle was a member of the American Association for the Advancement of Science, the Society of American Botanists, American Phytopathological Society (Charter and Life Member), American Society of Plant Taxonomists, Society of American Bacteriologists, and The Montana Horticultural Society (Life Member). He was also a member of Sigma Xi, Phi Kappa Phi, and Phi Sigma, is listed in "Who's Who in America" and in American Men of Science. In the field of botanical sciences he made notable and permanent contributions. His cytological studies concerning the "Formation of the Spores in the Sporangia of *Rhizopus nigricans* and *Phycomyces nitens*" have been quoted and copied by numerous authors of fundamental works in botany. He was the author of three important textbooks: "Textbook of Systematic Botany," "Plant Life," and "General Bacteriology," which have been adopted in many colleges and universities.

Dr. Swingle was loved, respected, and admired by students and faculty alike. His greatest satisfaction was derived from his association with students and in teaching, for he was above everything else a teacher.

His relation to his colleagues was always that of a friend and counselor. He never discredited the opinions of others, even though they differed from his own, but would offer suggestions that would often tend to prove his point. His interest reached far beyond his own particular sphere of endeavor, and

the success of any department was interpreted by him as lending success to his own department, as well as advancing the prestige of Montana State College.

Dr. Swingle believed in people. He found the finest delights of his experience in developing and furthering the interests of others. Although he saw their imperfections he never slackened his efforts to help them to be more nearly the men and women they were capable of being. His work was therefore conceived to be an opportunity for a service that would assure a continuing influence. In this way he developed the kindly, sympathetic, personal philosophy that so charmed his friends and acquaintances and laid the foundation for a true memorial—not in stone or steel, but in the hearts of those who knew him.

To those who knew him intimately the loss of his counsel, his sympathetic understanding, and his friendship will be keenly felt.



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RHIZOME TREATMENTS FOR CONTROLLING BOTRYTIS CROWN ROT IN IRIS[1]

LOUISE DOSDALL

(Accepted for publication February 28, 1944)

Botrytis convoluta was described by Whetzel and Drayton (7) in 1932 as a new species of fungus parasitic on rhizomatous iris. According to these authors the fungus had been intercepted during the ten years previous by United States Department of Agriculture Quarantine Inspectors on iris rhizomes from France, Germany, England, and Holland and had been observed in New York since 1924, in Washington state in 1931, and in Ontario, Canada, in 1927. In 1934 the disease was reported from Minnesota (2), in 1937 from New Jersey (8), in 1938 from British Columbia (1) and Idaho (in correspondence to the author), and in 1939 from Missouri (6); so that it seems to be quite generally distributed at least through the northern part of the United States. A basal gray mold caused by Botrytis species was reported in 1939 (5) as the third most important disease of iris in England, but it is not clear whether the fungus involved is *Botrytis convoluta.* Other references that might be interpreted as pertaining to this species are lacking in the European literature. In 1937 Drayton (3) described the ascigerous stage which he produced in culture and named *Sclerotinia convoluta.*

In the spring of 1934 Botrytis crown rot was very severe in several iris plantings in Minnesota, and in at least three nurseries continued cultivation of iris was threatened because of the destructiveness of the disease and by the great masses of sclerotia and conidia produced as potential inoculum. Rhizome treatments seemed one possible means of preventing spread of the disease and of establishing new plantings free from the disease. In late summer rhizomes from a miscellaneous collection of old varieties of iris were obtained for experimentation from a heavily infected planting that had been observed during the entire growing season of 1934. After the iris had started growth in the spring no further development of the rot was apparent. The fans which had not been killed during the winter grew vigorously and by midsummer there was nothing to indicate presence of the disease except the many missing plants in the rows. As the rhizomes were being cut for replanting, no signs were discovered by which a grower might detect the disease in his stock. With the material available rhizome treatments were planned to obtain as much information as possible concerning the extent to which infection is carried on the rhizomes and the extent to which fungicides are effective in eliminating the disease.

It was common practice among Minnesota iris growers to throw a handful of copper sulphate crystals around the crown of iris plants in the fall to

[1] Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 165–71–1–124, sponsored by the University of Minnesota. Paper No. 2142, the Scientific Journal Series, Minnesota Agricultural Experiment Station.

protect them from rot in general. Mercuric chloride was used to some extent as a rhizome dip to control bacterial soft rot. It was thought that calomel or Semesan might be less injurious, or formaldehyde, if effective, cheaper. These five fungicides were selected and used in the following strengths: mercuric chloride 1:1000, mercuric chloride 1:1000 plus one per cent hydrochloric acid; calomel 1:128 (one ounce to one gallon of water); Semesan 1:400 (0.25 per cent); copper sulphate 1:100; and formalin 1:80, 1:160, and 1:320. For each of the treatments five rhizomes of each of 13 varieties were available. After the rhizomes had been cut into one-fan units and trimmed according to the common nursery practice, each fan-unit with rhizome and the portion 2 to 3 in. above it was treated 5 minutes, drained on newspaper 30 minutes, and wrapped in dry paper until planted

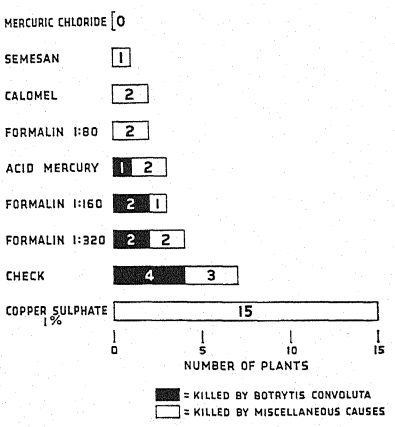


FIG. 1. Results in the spring of 1935 of treating iris rhizomes August 30, 1934, to control Botrytis crown rot. Each bar represents 65 plants belonging to 13 varieties.

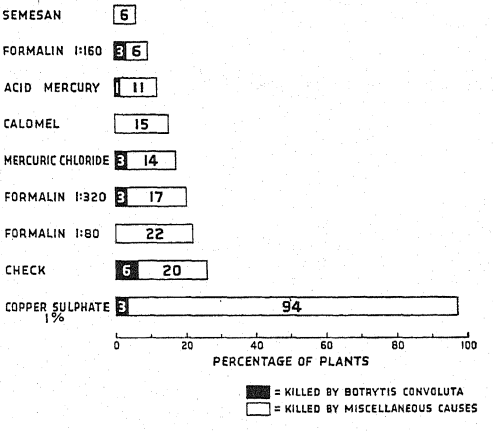


FIG. 2. Results in the spring of 1935 of rhizome treatments August 30, 1934, on the control of Botrytis crown rot in Monsignor, Malvina, and an unknown variety of iris.

five days later. During the winter the plants were covered with a straw mulch. The amount of disease in the plots was observed the following spring.

In the spring of 1935, only 23 (2 per cent) of the 585 plants in the experiment were infected with *Botrytis;* considering the checks alone, 4 plants (6 per cent) out of 65 carried the infection as evidenced by the presence of the fungus (Table 1 and Figs. 1 and 2). This low percentage of rhizomes carrying the disease limited the range within which the treatments exerted their effect. However, 20 per cent of the check plants were lost from miscellaneous causes. This loss was reduced to 17 per cent by formalin 1:320, to 15 per cent by calomel, to 14 per cent by mercuric chloride, to 11 per cent by the acid mercury, and to 6 per cent by both Semesan and the formalin 1:160 (Table 1 and Figs. 1 and 2). Thus these treatments exerted a beneficial effect that is not evident if one considers only plants rotted by *Botrytis* in the spring.

TABLE 1.—*The effects of rhizome treatments on Botrytis crown rot of iris*

Treatments[a]	Check			$CuSO_4$ 1:100			$HgCl_2$ 1:1000			$HgCl_2$ + HCl			Semesan 1:400			Calomel 1:128			HCHO 1:80			HCHO 1:160			HCHO 1:320			Total No. for each variety			Per cent for each variety		
Varieties	M[b]	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H
Miss Maggie	2	0	3	5	0	0	2	0	3	1	0	4	1	0	4	1	0	4	4	0	1	0	0	5	1	0	4	17	0	28	38	0	62
Shakespeare	3	0	2	5	0	0	0	0	5	0	0	5	0	0	5	1	0	4	0	0	5	0	0	5	1	0	4	10	0	35	22	0	78
Edith	0	0	5	3	2	0	0	1	4	0	0	5	0	0	5	0	0	5	1	0	4	0	0	5	2	0	3	6	3	36	13	7	80
Monsignor	2	2	1	5	0	0	0	0	5	1	0	4	0	0	5	0	0	5	1	0	4	1	0	4	1	2	2	11	4	30	25	8	67
Unknown	0	0	5	4	0	1	0	0	5	0	0	5	0	0	5	0	0	5	1	0	4	0	0	5	0	0	5	5	0	40	11	0	89
Rose Unique	2	0	3	5	0	0	2	0	3	1	0	4	0	0	5	1	0	4	2	0	3	0	0	5	4	0	1	17	0	28	38	0	62
Malvina	0	1	4	5	0	0	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	2	3	0	0	5	5	3	37	11	7	82
Gertrude	1	0	4	4	0	1	1	1	3	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	6	1	38	13	2	85
Eugene Sue	2	0	3	5	0	0	3	0	2	3	0	2	1	0	4	4	0	1	1	0	4	1	0	4	1	0	4	21	0	24	47	0	53
Unknown	1	1	3	5	0	0	0	0	5	1	1	3	1	0	4	2	0	3	1	0	4	0	0	5	1	0	4	12	2	31	27	4	69
Unknown	0	0	5	5	0	0	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	5	0	40	11	0	89
Unknown	0	0	5	5	0	0	1	0	4	0	0	5	1	0	4	1	0	4	3	0	2	2	0	3	0	0	5	13	0	32	29	0	71
Unknown	0	0	5	5	0	0	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	5	0	40	11	0	89
Total no. for each treatment	13	4	48	61	2	2	9	2	54	7	1	57	4	0	61	10	0	55	14	0	51	4	2	59	11	2	52	133	13	439	.....	.....	.....
Per cent for each treatment	20	6	74	94	3	3	14	3	83	11	1	88	6	0	94	15	0	85	22	0	78	6	3	91	17	3	80	0	0	0	23	2	75

[a] Rhizomes of 13 varieties treated August 30 and planted September 4, 1934. Observations May 7, 1935. All plants covered with straw during winter.

[b] Under M are the numbers of plants missing from miscellaneous causes; under B the numbers infected by *Botrytis*, and under H, the numbers remaining healthy.

Originally it was hoped to find some measure of the injury to the plants that might be caused by any of the treatments. Notes were taken in the fall on the condition of the plants just before the straw mulch was applied. At this time a few plants had disappeared. Some of the plants that seemed not to be growing at this time were in good condition the following spring and some that were good in the fall had disappeared in the spring. Unfortunately, from the nature of the disease, considerable time must elapse between rhizome treatment and observations on the effect of treatments in suppressing the disease, in this case from August 30, 1934, to April 1, 1935. During this period it is difficult under Minnesota conditions to follow the progress of the disease and to account for all the things that may happen to the plants. Some of the plants were lost by freezing during the winter. It was discovered later that some of the newly set rhizomes lost during hot dry periods in late summer or early autumn were rotted by *Rhizopus*. Some of the plants that rotted during the winter were inadvertently removed with the straw mulch in the spring. The best criterion of the effectiveness of the treatments therefore seemed to be either the percentage of healthy plants or the combined losses from *Botrytis* and from miscellaneous causes. Where the latter greatly exceeded that in the checks it was assumed to be due to injury from the fungicides; where the number of healthy plants greatly exceeded the number in the checks the benefits were assumed to be due to treatments. Whether all the beneficial effect was due to suppression of the Botrytis rot cannot be stated. The one per cent copper sulphate definitely injured the plants (Fig. 1). The formalin 1:80 and 1:320 plots were not much better than the check plots; and the lowest percentage of plants was lost in the Semesan plots.

Only three varieties, Monsignor, Malvina, and an unknown variety, carried the Botrytis infection in the checks (Table 1). Figure 2 shows the incidence of Botrytis infection in each of the treatments for the 15 plants concerned. This probably gives the better picture of the effect of the fungicides in suppressing the disease even though the number of plants concerned is small.

Experiments of the first year showed that the fungus was carried over from one field to another on the rhizomes to a limited extent. Rhizome treatments at transplanting time gave encouraging results as a control measure. In the three varieties with Botrytis rot in the check plots, mercuric chloride, Semesan, calomel, and formalin 1:80 eliminated the disease; the acid mercury and the weaker formalin solutions reduced the amount; but the copper sulphate 1:100 definitely killed the plants. The mercuries, therefore seemed to offer good prospects for control, formalin seemed deserving of further experimentation as to concentration and length of treatment, and it was apparent that the copper sulphate should be tested at a much reduced strength.

In the various iris plantings observed in the spring of 1934, the heaviest infections of Botrytis crown rot were in those plantings that had been cov-

ered with straw during the winter. To determine whether the rot develops more severely under a covering of straw or when the plants are exposed during the winter, half of 540 plants treated as in the previous experiment were covered with straw and half were not covered. Ten rhizomes of each of six varieties of iris were used for each treatment. One variety, in which no *Botrytis* appeared in either the covered or the not covered check plots, was not included in the summary of results in table 2. Of the 225 plants in the covered series 27 per cent were lost from miscellaneous causes and 2 per cent from *Botrytis,* while in the series where the plants were not covered 28 per cent were lost from miscellaneous causes and 9 per cent from *Botrytis.*

TABLE 2.—*The effects of straw mulch and rhizome treatments on Botrytis crown rot of iris*

Treatments[a]		Plants covered						Plants not covered					
		Killed				Healthy		Killed				Healthy	
		Misc. causes		*Botrytis*				Misc. causes		*Botrytis*			
Fungicide	Strength	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
Check		9	36	2	8	14	56	5	20	11	44	9	36
Copper sulphate	1:100	24	96	0	0	1	4	20	80	3	12	2	8
Mercuric chloride	1:1000	4	16	0	0	21	84	5	20	0	0	20	80
Acid mercury	1:1000	3	12	0	0	22	88	5	20	0	0	20	80
Semesan	1:400	1	4	0	0	24	96	6	24	1	4	18	72
Calomel	1:128	3	12	0	0	22	88	8	32	1	4	16	64
Formalin	1:80	8	32	0	0	17	68	6	24	0	0	19	76
Formalin	1:160	1	4	0	0	24	96	4	16	1	4	20	80
Formalin	1:320	7	28	2	8	16	64	3	12	3	12	19	76
Total		60	27	4	2	161	71	62	27	20	9	143	64

[a] Rhizomes of 5 varieties treated August 30 and planted September 4, 1934. Observations May 7, 1935.

Considering only the check plots, 9 plants in the covered series were lost from miscellaneous causes and 2 from *Botrytis,* a total of 11 of the 25 plants lost; while in the series which was not covered 5 plants were killed by miscellaneous causes and 11 by *Botrytis,* a loss of 16 of the 25 plants. Here again some of the plants killed by *Botrytis* may have been removed inadvertently with the straw mulch so that the effectiveness of the treatments under the two conditions is best depicted by the total loss of plants or by the number of healthy plants.

Figure 3 compares the percentages of plants lost in the two series of each treatment (25 plants). The upper bar of each pair represents the covered plot and the lower bar the plot which was not covered, while the solid portion (white or black) of each bar represents the plants killed by *Botrytis* and the shaded portion those killed by miscellaneous causes. Iris in the copper sulphate plots came through the winter poorly: only one plant escaped

killing in the covered series and three in the series not covered. In general, however, a straw covering reduced the loss of plants in both check and treated plots. The loss attributable to *Botrytis* seemed to be eliminated in most of the treated covered plots and to be greatly reduced in covered check plots. The losses resulting from miscellaneous causes were slightly or greatly reduced by straw covering plus certain of the treatments (mercuries and formalin at 1:160) but were not reduced with straw covering alone or with straw covering plus copper sulphate or formalin at 1:80 and at 1:320. Rhizome treatment alone, without straw covering, always reduced or eliminated the loss from *Botrytis* but seldom reduced the losses from miscellaneous causes.

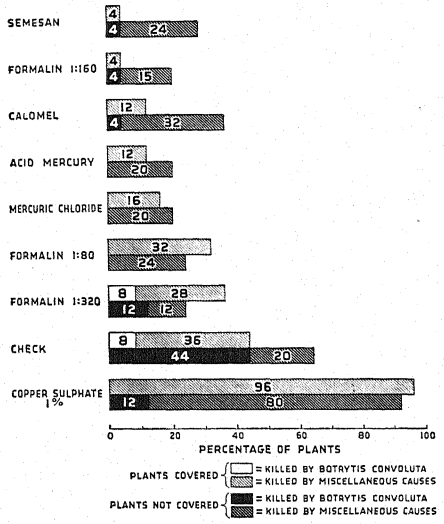


FIG. 3. Comparison of the amount of Botrytis crown rot developed from treated iris rhizomes with and without a straw covering during the winter 1934–1935. Each bar represents 25 plants belonging to 5 varieties.

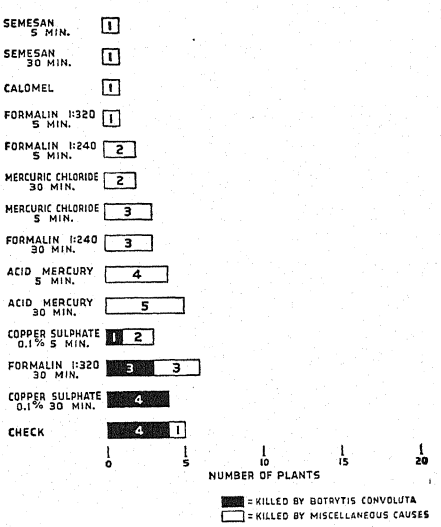


FIG. 4. Results in the spring of 1936 of rhizome treatments October 1, 1935, on the control of Botrytis crown rot. Variety Labor.

Thus under the conditions of this experiment, fewer plants were lost from Botrytis crown rot when the plants were covered with a straw mulch than when they were left exposed during the winter.

In the fall of 1935 it was possible to obtain from a private nursery a quantity of rhizomes of the variety Labor. These plants had been divided and set out in the late summer of 1933. In the spring of 1934 there had been considerable loss from Botrytis rot although exact counts on the percentage of plants infected were not made. Frequently several adjacent plants in a row were rotted by *Botrytis,* then for a distance all the plants were healthy. In some cases an entire row had been killed. The spotted distribution of the diseased plants in this field particularly had suggested that the infection was carried to the field on the rhizomes. This planting was reported to have very little Botrytis rot in the spring of 1935.

When the rhizomes of these plants were cut into one-fan units for replanting they were perfectly sound and there was no striking evidence of disease. The field evidence seemed to be that the disease had disappeared from the planting. The outer leaves, however, were dry on almost all the fans and at the base of the leaves there was a leathery dry rot similar to that from which *Botrytis convoluta* was isolated in the fall of 1934. No tissue cultures were made from this material, however. These outer leaves did not separate and leave a clean scar as do normally senescent leaves; they tore irregularly, leaving fragments of dead leaf tissue attached to the rhizomes.

The same method of rhizome treatment was followed as in 1934. The strength of the copper sulphate solution was reduced to 1:1000, the formalin 1:80 was omitted and a 1:240 was substituted for the 1:160. For all treatments except the calomel, 5-minute and 30-minute immersion periods were tested. Twenty rhizomes were used for each treatment. The rhizomes were treated October 1, 1935, and planted October 2, in two blocks with the 14 treatments randomized in each. Final notes on the number of plants killed by miscellaneous causes, killed by *Botrytis,* and infected by *Botrytis* but not killed were recorded May 13, 1936. All of the plants were covered with straw during the winter. More accurate counts than in the previous year of the number of plants killed by *Botrytis* were obtained by examining each individual plant as the straw was removed from it.

The results are summarized in figure 4. Each bar represents the number of plants lost, the clear portion those lost from miscellaneous causes and the black portion those from *Botrytis.* One plant in the 30-minute copper sulphate plot was infected with *Botrytis* but was not killed. In 1937 this same plant developed no signs of the disease. In all other cases the plants showing signs of *Botrytis* were dead in the spring of 1936. No *Botrytis* appeared in any of the plots except the checks, the two copper sulphate and the 30-minute formalin 1:320 treatments. The best stands were obtained with the two Semesan, the calomel, and the five-minute formalin 1:320 treatments. With the 30-minute acid mercury and the 30-minute formalin treatments as many plants were lost as in the check plots. The significance of these results was evaluated by calculating the analysis of variance for the number of living plants in each plot since the majority of the plots contained no plants with signs of Botrytis infection. With regard to stand there were no statistically significant differences in the response of the rhizomes to the various treatments. However, from the standpoint of elimination of the disease the results were consistent with those of the previous year, namely, the mercury treatments gave good promise of adequate control, the formalin fair promise that the proper combination of strength of solution and period of immersion might be worked out, and the copper sulphate seemed unsatisfactory.

In the summer of 1937 rhizomes of the plants set out in 1934 were available for experimentation. This block of plants had been under continuous observation since planted. In the spring of 1937 a high percentage of

plants was diseased as a result of inoculations with macroconidia of *Botrytis convoluta* the previous November. Moreover, Botrytis rot resulting from natural infection was extremely heavy in the spring of 1937. This planting consequently offered the most uniformly infected lot of plants that had been available for study. During April and May each clump of iris infected with *Botrytis convoluta* was marked with a stake. The rhizomes from these plants were used in the late summer for further rhizome treatment experiments.

In spite of the heavy infection of Botrytis rot in the spring, the new rhizomes in late summer showed no signs of disease. In some varieties the soil close to the roots contained many black sclerotia of *Botrytis convoluta* which sometimes adhered very closely in the crevices between the roots and rhizomes. These sclerotia were particularly abundant among the roots of the variety Miss Maggie.

The same general method of treatment was followed as in the two previous years. The strength of the formalin solutions was changed to 1:200 and 1:400. A dry and a wet sulphur treatment were added to the list. In the dry treatment the rhizomes were shaken in a paper bag with a small quantity of dusting sulphur, in the wet treatment they were dipped in a suspension of wettable sulphur containing one pound sulphur in a gallon of water. Mercuric chloride and calomel were used in combination as well as singly. A yellow mercury oxide treatment was included also. Both the calomel and the yellow oxide were used simply in water suspensions and combined with gum arabic.

For making the gum arabic suspensions the recommendations of Glasgow (4) for the use of calomel in treating cabbage seed were followed. One part of gum arabic was ground as finely as possible in a porcelain mortar, a small amount of water added and ten parts of the mercury powder ground in to make a smooth paste. Finally enough water was added to give a proportion of one ounce of calomel or yellow oxide to one gallon of water. The gum held the calomel in suspension for several hours but in 24 hours it had settled out. The yellow oxide and the gum combination was not much more stable than the yellow oxide alone.

Twenty rhizomes of each of five varieties received each treatment on August 27, 1937, and were planted the following day in 2 randomized blocks in soil which had not grown iris previously. All the plots were covered with straw during the winter. Final counts on the number of plants infected with *Botrytis,* the number of plants killed by miscellaneous causes, and the number of healthy plants were recorded May 23, 1938. From these data the response of the iris rhizomes to the various treatments was again studied by calculating the analysis of variance for the number of living plants free from Botrytis infection.

The results are summarized in table 3. Examining the mean numbers of healthy plants for all the varieties, it is seen at once that all mercury treatments except the 10-minute Semesan gave highly significant superior

TABLE 3.—*Results of rhizome treatments to control Botrytis crown rot. Rhizomes treated August 27 and planted August 28, 1937; observations May 23, 1938. All plants were covered with straw during the winter*

Treatment			Mean No. healthy plants per plot of 10 plants					
Fungicide	Strength	Time in minutes	All varieties	Miss Maggie	Shakespeare	Edith	Monsignor	Columbia
Calomel	1: 128	10	**9.7	**9.5	10.0	10.0	*9.5	9.5
Calomel and gum arabic	1: 128	10	**9.5	**9.5	10.0	9.0	*9.5	9.5
Mercuric chloride and calomel	1: 1000	30	**9.4	**9.0	9.5	9.0	*10.0	9.5
Yellow mercury oxide and gum arabic	1: 128	10	**9.4	**8.5	9.0	9.0	*10.0	10.0
Yellow mercury oxide	1: 128	10	**9.3	**9.0	9.5	9.0	*10.0	9.5
Mercuric chloride and calomel	1: 1000	10	**9.2	**9.5	9.5	8.5	*10.0	8.5
Mercuric chloride	1: 1000	30	**9.1	*8.0	9.5	9.0	*9.5	9.5
Semesan	1: 400	30	**9.0	7.0	8.5	10.0	*9.5	10.5
Mercuric chloride	1: 1000	10	**8.7	*7.5	9.5	8.5	*9.0	9.0
Acid mercury	1: 1000	30	**8.6	7.0	8.0	9.5	*9.5	9.0
Acid mercury	1: 1000	10	**8.5	6.5	9.0	9.5	*8.5	9.0
Formalin	1: 200	30	*7.8	3.5	8.0	9.0	*9.5	9.0
Formalin	1: 400	10	7.3	5.5	8.0	8.5	5.5	9.0
Semesan	1: 400	10	6.7	4.5	6.0	8.5	7.0	7.5
Sulphur—wettable	1: 8	10	6.7	5.0	7.0	8.5	5.0	8.0
Formalin	1: 400	30	6.6	2.0	9.0	8.0	6.5	7.5
Formalin	1: 200	10	6.5	4.5	6.5	8.5	5.5	7.5
Sulphur—dusting	.....	.....	6.4	4.0	6.0	7.0	5.5	9.5
Check	.....	.....	6.5	4.0	8.0	8.0	5.0	7.5
Mean No. healthy plants per plot			8.15	6.53	8.45	8.78	8.13	8.87

	at 5 per cent level	at 1 per cent level
Difference necessary for significance between treatments	1.1	1.5
Difference necessary for significance between treatments within any variety	3.45	5.71
Difference necessary for significance between varieties	0.57	0.94

* = Significant difference between treatments at the 5 per cent level compared with the check.

** = Significant difference between treatments at the 1 per cent level compared with the check.

stands when compared with the checks and that the 30-minute formalin 1:200 was significantly superior to the check when compared at the five per cent level. The three other formalin treatments and the sulphur treatments were not significantly superior to the checks. There were no signifi-

cant differences between the effective mercury treatments except between the calomel treatment with the highest and the 10-minute acid mercury with the lowest mean. Gum arabic added nothing to the effectiveness of the calomel or yellow oxide of mercury, nor was the combination of calomel and mercuric chloride more effective than either fungicide alone.

Considering the mean numbers of plants for each variety, there is a significant difference in the response of the variety Miss Maggie to the treatments in comparison with the rest of the varieties even at the one per cent level. There is also a significant difference between the response of the variety Monsignor and varieties Columbia and Edith at the five per cent level. The Botrytis infection in the check plots was 50 per cent in the variety Miss Maggie, 15 per cent in Shakespeare, 15 per cent in Edith, 50 per cent in Monsignor, and 25 per cent in Columbia. The loss of plants from miscellaneous causes in the check plots was 10 per cent in Miss Maggie, 5 per cent in Shakespeare, 5 per cent in Edith, and none in Monsignor and Columbia. Thus, the varieties Miss Maggie and Monsignor carried considerably more Botrytis infection on the rhizomes than did the others and in the variety Miss Maggie an additional 10 per cent of the plants were lost from miscellaneous causes.

Examining the response of the individual varieties to the various treatments it is seen that significant differences between the checks and the treatments were obtained in the two varieties Miss Maggie and Monsignor, with the most marked response in the former. In the latter variety the acid mercury treatments and the 30-minute Semesan treatment as well as the 30-minute formalin 1 : 200 gave no significant differences in comparison with the checks, while the mercuric chloride treatments were significantly superior to the checks only at the five per cent level. In another experiment, however, with this same variety, where 40 rhizomes were used for each treatment, both the mercuric chloride treatments were significantly superior to the checks at the one per cent level.

In the tests of the two previous years there seemed to be considerable difference in the number of fans per plant in the different plots. It was thought that this might be an indication of a stimulatory or injurious response of the rhizomes to the various treatments or a varietal characteristic. A record was accordingly kept of the number of fans produced by each plant and the analysis of variance calculated for this character. No statistically significant differences were found for either the treatments or the varieties.

Since the plot from which the iris were taken in the late summer of 1937 had been so heavily infested with *Botrytis convoluta* in the spring, it offered a good opportunity to test the effect of the fungicides against soil-borne infection. A series of treated rhizomes paralleling the series planted in new soil was planted in the infested soil. After the iris had been removed from the field the soil was plowed, disced and harrowed. In replanting, the new rows were laid out in as nearly the same position as the former rows as was

possible. Sufficient space was available for six blocks of treatments similar to those planted in the new soil. There were rhizomes enough of three of the varieties used for the treatments in new soil, namely varieties Shakespeare, Edith, and Monsignor, to plant one series of 10 rhizomes for each treatment in the infested soil, or 190 rhizomes of each variety. The other three blocks were filled with three different varieties. The iris were dug August 31, the rhizomes treated September 2 and replanted September 3,

TABLE 4.—*Effect of rhizome treatment on Botrytis crown rot in iris planted in new and in infested soil, winter of 1937–1938*

Treatment		New soil (per cent plants)[a]			Infested soil (per cent plants)		
Fungicide	Time	Healthy	Killed by misc. causes	Infected by Botrytis	Healthy	Killed by misc. causes	Infected by Botrytis
Calomel	10	97	0	3	70	0	30
Calomel and gum arabic	10	95	0	5	74	3	23
Mercuric chloride and calomel	30	94	2	4	67	2	31
Yellow mercury oxide and gum	10	94	2	4	72	3	25
Yellow mercury oxide	10	93	1	6	73	0	27
Mercuric chloride and calomel	10	92	2	6	68	2	30
Mercuric chloride	30	91	2	7	57	2	41
Semesan	30	90	0	10	50	10	40
Mercuric chloride	10	87	6	7	59	3	38
Acid mercury	30	86	3	11	67	3	30
Acid mercury	10	85	3	12	38	8	54
Formalin 1: 200	30	78	4	18	54	8	38
Formalin 1: 400	10	74	3	23	45	0	55
Sulphur—wettable	10	67	4	29	50	3	47
Semesan	10	67	5	28	43	7	50
Formalin 1: 400	30	66	5	29	32	7	61
Formalin 1: 200	10	65	3	32	54	8	38
Sulphur—dusting	.....	64	8	28	38	12	50
Check	.....	65	4	31	32	15	53
Average		81	3	16	55	5	40
Average of the 3 varieties in both plots		84	3	13	53	7	40

[a] Percentages in the new soil columns are based on 100 plants. Percentages in the infested soil columns are based on 60 plants.

1937. On March 21, 1938, while the ground was still frozen, some of the straw covering the plants was lifted and *Botrytis convoluta* conidia were found on several plants. Final observations on the amount of the disease in the field were made May 24, 1938.

A comparison of the results obtained from this planting of treated rhizomes in infested soil with those obtained from planting treated rhizomes in clean soil is given in table 4. Percentages are tabulated of the plants living in spring but not infected with *Botrytis,* of the plants killed by miscella-

neous causes but not attributable to *Botrytis* directly, and of the plants showing definite signs of infection by *Botrytis*. The fungicides are listed serially in a descending scale according to the percentage of healthy plants in the new soil. According to this scale, the mercury treatments fall at the upper end and the formalin, sulphur, and check treatments at the lower. The percentages of plants killed by miscellaneous causes and the percentages of plants infected by *Botrytis* in general fall in the reverse order. This would indicate again that some of the unaccounted for loss of plants was actually due to factors controlled by the treatments, in this experiment perhaps most of it. The percentages of healthy plants run in almost the same sequence in the two types of soil but they are considerably lower in the new soil. The percentage of plants infected by *Botrytis* is from two to ten times as great for plots in infested soil as for the corresponding ones in new soil. In general, the mercuries tend to suppress Botrytis rot, but rhizome treatment is of little practical importance when the rhizomes are planted in heavily infested soil.

This relative effectiveness of rhizome treatment for three varieties in infested soil and in new soil is represented more strikingly in figure 5. Diseased and missing plants in new soil are represented by white bars and the plants diseased and missing in the infested soil by black bars, the solid portion of the bar in each case representing the percentage of plants infected by *Botrytis* and the shaded portion those lost by miscellaneous causes. The percentage in each white bar is thus based on 60 plants and the percentage in each black bar on 30 plants. For ease of comparison the white bars were superimposed on the black bars. Treatments are ineffective when the rhizomes are planted in infested soil except that there is a tendency for the fungicides effective in new soil to suppress the infection in infested soil. Calomel and gum arabic is considerably more effective in suppressing Botrytis crown rot in infested soil than calomel alone is. In all conclusions drawn from this experiment, however, it must be borne in mind that the pathogen was probably not distributed evenly through the soil in the infested plot since all of the iris in the previous planting were not infected. The plowing, discing and harrowing between plantings helped to distribute whatever sclerotia, spores and mycelium were in the soil but probably not so evenly as would be desired for a careful analysis. To compensate for this heterogeneity a large number of replications would have been desirable.

Finally, an attempt was made to determine whether rhizomes from plants visibly infected with *Botrytis convoluta* in the spring carried the infection more often than did those from plants in the same field showing no such signs of spring infection. In the spring of 1937 each clump of iris on which were found macroconidia, sclerotia, or the characteristic tan felty rot of the fungus was marked with a stake. In the late summer the rhizomes from these plants were harvested separately. For the variety Miss Maggie 400 rhizomes from the plants with signs of infection and 400 from plants with no signs of infection were subjected to the same series of treatments as those

used in the other experiments of this same year and then planted in new soil. Of these 800 rhizomes, 31 per cent of those from plants showing visible signs of *Botrytis* in the spring of 1937 were infected with *Botrytis* in the spring of 1938 and 26 per cent from plants with no signs of infection in the spring of 1937 were infected in 1938. Considering only the untreated rhizomes (40 in each case) 50 per cent from the plants with signs of infection in 1937 were visibly infected in 1938 and 40 per cent from the plants not visibly infected in 1937 were visibly infected in 1938.

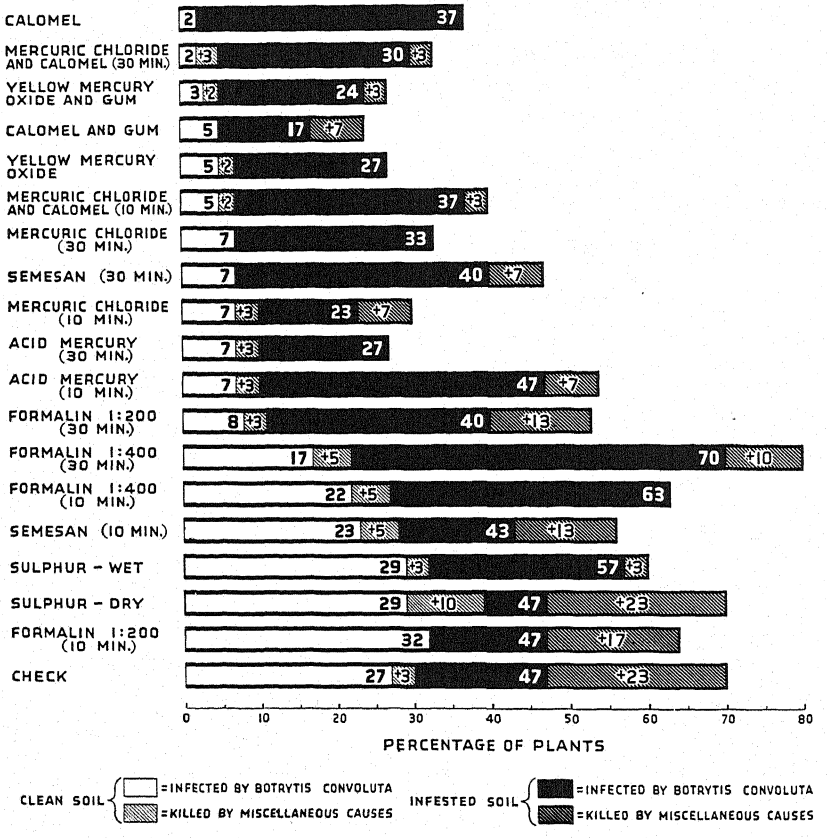


FIG. 5. Comparison of the effect of planting treated rhizomes in infested and in noninfested soil.

An analysis of variance was made for the number of healthy plants in the series of treated plots. There was no statistically significant difference between the number of healthy plants obtained from the rhizomes from infected plants and those from plants not infected. An analysis was also made comparing the number of plants visibly infected with *Botrytis* in the spring of 1938 from rhizomes of plants bearing visible signs of the fungus in the spring of 1937 and from plants bearing no such signs. The differences were not statistically significant. The conclusion is that when rhizomes are

taken from a planting of iris heavily infested with *Botrytis convoluta,* apparent freedom from disease in the spring is no assurance that the rhizomes will not carry the pathogen to a new planting.

In the late summer of 1939 the mercury treatments were tested once more, since these had given the most promising results in 1934, 1935, and 1937. Calomel, yellow mercury oxide, mercuric chloride, and Semesan were used. The first two fungicides were used again at the rate of one ounce to one gallon of water (1:128) both with and without the gum arabic. In the gum combinations twice the amount of gum, 20 per cent the weight of the chemical, used in 1937 was tested in an attempt to make the suspensions more stable. In these proportions the yellow oxide remained in suspension somewhat better than the calomel. This was exactly the reverse of the

TABLE 5.—*The effects of rhizome treatments with mercury fungicides on Botrytis crown rot in the iris variety Edith*

Treatment[a]			Mean No. healthy plants (20 replicates)	Percentage of plants		
Fungicide	Strength	Minutes		Healthy	Killed by misc. causes	Infected by *Botrytis*
Semesan	1: 200	30	4.80	96	1	3
Calomel and mercuric chloride	1: 128 1: 500	30	4.75	95	2	3
Calomel	1: 128	Dip	4.70	94	1	5
Mercuric chloride	1: 500	30	4.60	92	3	5
Yellow mercury oxide	1: 128	Dip	4.55	91	2	7
Calomel and gum	1: 128	Dip	4.50	90	4	6
Yellow oxide and gum	.....	Dip	4.45	89	4	7
Check	.....	.....	4.05	81	5	14

Difference between means necessary for significance at the
5 per cent level = 0.036
1 per cent level = 0.047

[a] Rhizomes treated and planted September 14, 1939. Observations May 21, 1940.

experience in the previous trials. Mercuric chloride and Semesan were used at twice the strength used before and with only the 30-minute soak for each.

Rhizomes of only one variety of iris, Edith, were used in this experiment. The plants from which these were taken had been in the field two years. In the spring of 1938, 27 per cent were infected with *Botrytis convoluta* and in the spring of 1939, 18 per cent. The plants were dug September 6 as they occurred in the field without any attempt to select only those plants which bore visible signs of the fungus the preceding spring. At cutting time there was no decay in the rhizomes but in many the outer leaves were dry and tore irregularly from the rhizome. One hundred one-fan units were used for each treatment. The rhizomes were treated in the field on September 14 and planted immediately after being taken from the solutions.

One hundred rhizomes were treated together, then separated into lots of five, and planted in 20 different blocks. The eight treatments were randomized within each block. Thus there were 20 replications for each treat-

ment. Final records of the number of living plants free from visible signs of Botrytis infection, the number of plants dead or missing from causes not attributable to *Botrytis* directly, and the number of plants visibly infected or killed by *Botrytis* were made May 21, 1940.

To test the effectiveness of the various treatments an analysis of variance (Table 5) was made for the number of living plants in the spring of 1940 free from visible signs of Botrytis infection. In the check plots 14 per cent of the plants were infected with *Botrytis convoluta* and 5 per cent were killed by miscellaneous causes. Thus the rhizomes carried a relatively low amount of infection. From a comparison of the mean number of healthy plants per plot all of the treated plots produced a stand significantly better than the check and the individual treatments can be rated for effectiveness in the order of their means. Gum arabic decreased the effectiveness of both the calomel and the yellow oxide of mercury. The calomel and mercuric chloride treatment was statistically superior to the calomel alone but the one per cent increase in stand would scarcely be of practical significance. In this same variety, with rhizomes carrying the same amount of infection there were no significant differences between the mercury treatments in the 1937 experiment when only 20 rhizomes were used for each treatment.

DISCUSSION

Along with the experiments on treating rhizomes to control Botrytis crown rot other studies were undertaken to learn more of the nature of the disease, its development from year to year under known field conditions and its destructiveness over a period of years. Under the climatic conditions which prevail in Minnesota Botrytis crown rot is primarily a disease of the dormant plants. The fungus itself, in relation to the iris plant, appears to be dormant or inactive during the summer. In September or October, in some years, depending upon weather conditions, it may be found sporulating inconspicuously at the base of the outer leaves or old flower stalks. In other years there are no signs of the disease in fall. Sclerotia too have been found forming in October on the cut surfaces of newly divided rhizomes. In general, however, at the time the iris are covered with the usual straw mulch in October or November, or when the natural snow covering falls, the plants appear in excellent condition. At the time the covering is removed in March or April many plants may be rotted and quantities of sclerotia and conidia found on the plants.

The period of maximum activity of the fungus varies greatly from year to year depending apparently on weather conditions. In some years practically all the damage is done under the straw or snow covering. If the weather is warm and dry in April so that the iris start growing rapidly, no further damage is done. If, on the other hand, it is cool for several weeks, or months even, the rot will continue to develop and sclerotia and conidia will be formed in great abundance. Thus under Minnesota conditions the activity of the fungus in the plant may cease suddenly in March or it may continue into June.

It is only during this period in spring that the disease is easily detected in an iris planting. Yet it is mostly overlooked, because the plants which fail to develop are considered by the average grower to have been lost through winter injury. The mass of conidia at the base of the plant is such a neutral grayish brown that it is difficult to distinguish from the surrounding soil. Then, too, most of the evidence of the disease is removed with the covering or in cleaning away the dead leaves. When iris are divided in late summer there are no easily detected signs of the disease, so that the necessity of treatment at this stage is not recognized. The fact that the causal agent is carried on rhizomes which show no signs of infection and even on rhizomes which come from plants showing no signs of infection in spring is of importance to the individual purchaser of iris and to the nursery grower.

The severity of the disease varies greatly from year to year even in the same planting. A high percentage of loss one year may be followed the next with only a trace of the disease in the same field. However, in every planting in which the disease has been found, it has persisted from year to year unless special measures were taken to eliminate it. The apparent absence of the disease for a year or two, or even for several years, gives the impression that the disease is not serious and tends to disappear of itself and also leads the grower to minimize the need of control measures when they will be most effective.

The ineffectiveness of the rhizome treatments in infested soil makes evident the necessity of an adequate system of crop rotation combined with effective rhizome treatments. That these measures have given practical results has been demonstrated by nursery growers. One grower who has very carefully cleaned up his stock has obtained circumstantial evidence that the disease is more widespread in the United States than survey reports would lead one to believe, since rhizomes received from points extending to the east coast, the west coast and as far south as Tennessee have developed the disease when planted in his nursery without rhizome treatment while his own stock remained free from the disease. He now has made it a regular practice to treat all stock brought into his nursery from the outside.

Rhizome treatment has proved to be a more complex problem than simply finding a satisfactory fungicide and brings up a number of questions which need more careful attention. There seems to be a marked difference in the frequency with which rhizomes of some varieties carry the infection, although on individual plants the disease seems to be just as destructive in one variety as in another. Is this an indication of a type of resistance and how and where is the fungus carried on the rhizomes? Is rhizome treatment more necessary for some varieties than for others? Also, different varieties respond differently to the same treatments. The marked failure of the variety Miss Maggie to respond to the 30-minute Semesan and the acid mercury treatments in the 1937 experiments brings out the necessity for the study of the behavior of fungicides in relation to specific varieties. The

relationships between various types of winter covering and the development of Botrytis crown rot as well as the protection these coverings provide the treated rhizomes need further study. The four most destructive occurrences of the disease that have been observed were all in areas which had been covered with several feet of snow during the winter.

While the mercury treatments in general gave good results, the wholly unsatisfactory tests with copper sulphate by no means eliminate the possible effectiveness of other copper preparations. Systematic experimentation with formaldehyde solutions might lead to adequate procedures, although the nicety of balance between the strength of solution, the length of treatment and the variety of iris treated will evidently be finer than with the mercuries. In addition to the results of the experiments recorded, the experience in one nursery in the first years of the study seems to indicate that formaldehyde even in dilutions too weak to be effective burned the foliage of some of the varieties to such an extent that the plants did not become established before cold weather set in. The possibilities of many other compounds are not to be overlooked.

The general conclusions to be drawn from this study are that *Botrytis convoluta* has high potentialities as a destructive agent in iris plantings. Because the greatest damage is done during the dormant period of the host and the signs of the disease are inconspicuous, it is usually overlooked and the injury is dismissed as winter killing. Even though the plants are apparently unaffected during the growing season and there are no signs of decay on the rhizomes at transplanting time, rhizomes from an infested planting nevertheless carry the infection which, however, does not make itself manifest until the following spring. With the extensive interchange of rhizomes that takes place the implication is that the disease must occur practically wherever iris are grown. The disease may cause greater damage in some regions than in others but wherever many iris are lost by so-called winter killing a more careful study should be made to determine how much of this is really due to *Botrytis convoluta* and how much can be eliminated by rhizome treatment.

SUMMARY

During four years 5330 iris rhizomes belonging to 14 varieties were treated in various ways to determine to what extent *Botrytis convoluta* is carried on the rhizomes and whether the fungus can be eliminated by rhizome treatment.

Without any outward signs of disease, rhizomes from an infested planting carry the pathogen to a varying degree depending upon the season and apparently upon the variety.

With rhizomes from a heavily infested planting, no significant differences were found in the number of rhizomes carrying the infection, whether from plants bearing conidia, sclerotia or typical rot in the spring or from plants with no such signs of the disease.

The disease is easily overlooked and the loss of plants attributed to winter injury.

In one year's test the disease was much less severe under a straw covering than without a covering.

Rhizome treatments at transplanting time effectively eliminated the disease if the rhizomes were planted in clean soil.

Various mercury treatments, calomel, Semesan, yellow oxide, mercuric chloride, acid mercury, were all effective.

Some of the formalin treatments were as effective as the acid mercury but further trials are needed before a satisfactory combination of strength of solution and period of immersion can be worked out for all varieties.

Copper sulphate and sulphur in the combinations used were wholly unsatisfactory.

All treatments tested were ineffective in heavily infested soil, although a calomel suspension stabilized with gum arabic gave some promise of effectiveness.

Varieties responded differently to the same treatments.

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LIMA BEAN SEED TREATMENT ON LONG ISLAND[1]

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INTRODUCTION

Lima beans constitute one of the major crops grown on Long Island. The "Fordhook" is used exclusively in commercial plantings and for that reason the work reported in this paper was confined to that variety.

Most of the Limas are grown on soil which is too light for profitable potato production, although a considerable acreage is also grown as a second crop following potatoes. Long Island soils are generally well drained and naturally acid in their reaction. It is rare to find a bean field with a soil reaction higher than pH 5.4 and more often it is well below this figure.

Seed treatment in some form is commonly used with many vegetables as a means of protection against pathogens during the pre-emergence period. With some vegetables seed treatment can be relied upon to improve the stand and less seed per acre can be recommended when treatment is practiced. Until recent years, however, seed treatment has not been recommended for Lima beans.

Work done by Cunningham and Sharvelle (1) indicated that treating with certain organic materials, one of which later was marketed under the trade name of "Spergon," might prove effective in protecting Lima-bean seed under adverse conditions during the pre-emergence period. Later work by McNew (3) has shown that, under western New York conditions, seed treatment of Limas results in improved stands and increased yields. Recently Leach and Holland (2) have reported that seed treatment greatly improves the stand of Lima beans under California conditions.

On Long Island, particularly with early plantings, poor stands of Lima beans are the rule rather than the exception. An effort was made to find some dependable seed protectant which could be recommended for use with this crop.

METHODS

All of the materials used in the experiments were in the form of dusts and were used at the rate of 1.5 ounces per bushel of seed, with the single exception of yellow Cuprocide which was used at half that dosage.

Small plats were used in all tests made at the Research Farm, Riverhead, L. I. The number of seeds per plat and the size of the plat varied in different seasons, depending upon the number of materials used and the amount of land available. In no case was fewer than 50 seeds planted in each plat. With the exception of the 1943 tests all seed was planted by hand with uniform spacing in the row. These small plat tests were used

[1] Journal Paper No. 589, New York State Agricultural Experiment Station, Geneva, N. Y.

as a basis for determining the value of any given material as a seed protectant for Lima beans.

For three years this work was supplemented by machine-planted tests on farms of growers in Suffolk county, on the eastern end of Long Island. These tests were confined to such materials as showed some promise as a seed protectant and were available on the market for the use of growers. In

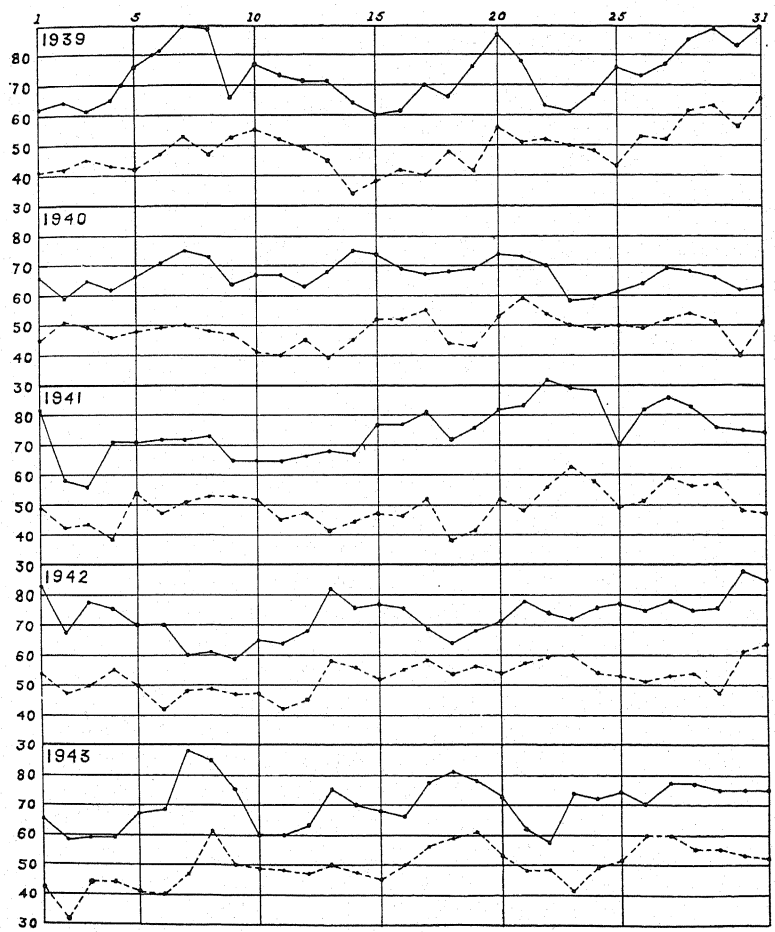


FIG. 1. Maximum and minimum temperature during May, 1939–43 inclusive.

1939 these tests were single-row plats, but in 1940–41 they consisted of half-acre contiguous plats in the same field.

The value of the data was determined by the analysis of variance method. Because of the nonuniformity of size of plats and rate of seeding the stand data are given in per cent rather than actual stand. Yield data are expressed in bushels per acre. In both cases the data represent the average of the number of replications used in the tests. Significant figures are marked with an asterisk.

RESULTS AND DISCUSSION

Any experimental work done under field conditions and covering a period of years will of necessity be carried on under varying climatic conditions. Seasonal conditions vary from year to year and planting dates are subject to local influences. For this reason it is necessary to consider climatic factors in this discussion and the data given indicate that there is a very definite relation between these factors and the results obtained from seed treatment of Lima beans.

The amount of moisture present in the soil at planting time, which is in itself dependent upon rainfall, plays an important role in germination and emergence of Lima beans under Long Island conditions where shallow planting is the general practice. High winds are common and the soil dries rapidly. It is not uncommon to see a marked difference in the time of emergence in a portion of a field planted in the morning as compared with a portion of the same field planted in the afternoon, a difference which can only be accounted for by loss of soil moisture during the day. Low soil moisture at planting time delays germination and emergence. Rainfall immediately following planting, provided it is not too heavy, will offset this condition to some extent but the longer the time elapsing between planting and the first rainfall, the longer will be the pre-emergence period.

The planting season on Long Island is from about May 1 to July 10 and at any time during this period the number of days necessary for emergence is largely dependent upon temperature and rainfall. During the first week in May the temperature (Fig. 1) is normally too low for quick emergence and a period of two weeks or more may elapse between the time of planting and the appearance of seedlings. Later in the season the temperature is usually sufficiently high for rapid germination and emergence. Low soil moisture at planting time, followed by inadequate rainfall, invariably lengthens the pre-emergence period regardless of temperature. The pre-emergence period will also be lengthened if the soil becomes packed because of too heavy rainfall. Observation over a period of years has shown that the ultimate stand of Lima beans is reduced by any delay in emergence and that both temperature and rainfall have a direct bearing on the results obtained from seed treatment.

At the Research Farm, with the exception of the year 1939, no significant difference in stand was obtained from seed treatment in plantings made after June 1. In all of these late plantings the soil moisture was either ample at planting time or rain fell shortly after planting and the temperature was high.

The year 1939 was one of the driest on record. At the time of the first two plantings soil moisture was low and subsequent rainfall was light (Fig. 2). Under these conditions both Spergon and Semesan Jr. significantly increased the stand. At the time of the last planting the soil moisture was at a somewhat higher level but the temperature was high and no

rain fell for eleven days after planting. All of the materials used were effective under these conditions and the increase in stand was highly significant (Table 1).

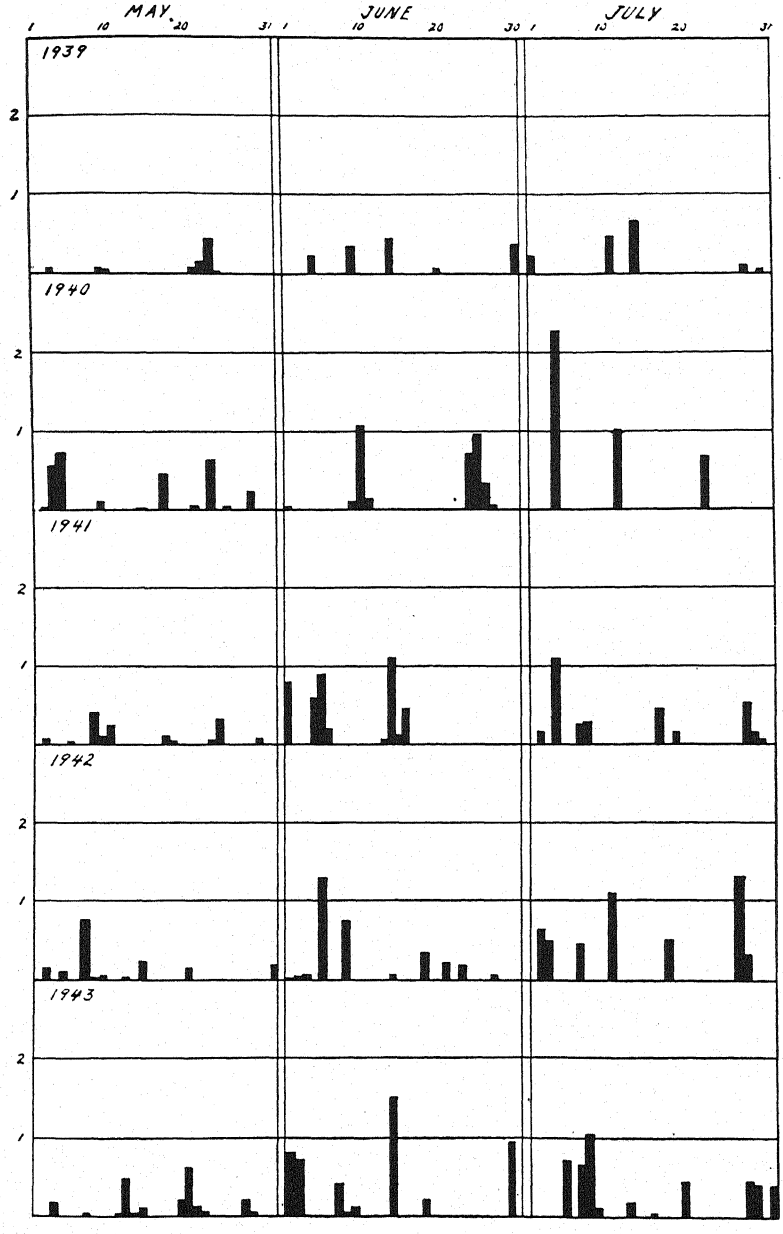


FIG. 2. Rainfall in inches for May, June, and July, 1939–43 inclusive.

On farms of growers in 1939 the results were not so favorable for seed treatment (Table 2). These tests were in widely scattered localities and

TABLE 1.—*The percentage stand and the yield in bushels an acre of Lima beans after seed treatment, at Research Farm, Long Island*

Date planted	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Number of replicates	
1939	Not treated		Spergon		Semesan Jr.		Red Cuprocide		Yellow Cuprocide		Yield	Stand
May 8	67	62	84*	111*	78*	65	74	80*	58	48	4	4
June 3	53	45	72*	68	75*	55	49	43	37	28	4	4
July 1	33	9	85*	41*	86*	17	82*	25*	.....	.....	4	4
1940	Not treated		Spergon		Semesan Jr.							
May 13	51	.....	60	.....	49	.....					4	...
29	63	.....	83*	.....	79*	.....					4	...
June 7	52	32	67	31	69	36					4	4
12	81	43	89	76	81	51					4	4
20	89	130	91	163	95	148					4	4
July 3	87	157	87	147	87	156					4	4
1941	Not treated		Spergon		Semesan Jr.		Copper oxychloride sulfate					
May 6	74	209	91*	204	92*	188	67	136			4	4
16	90	146	95	177	97*	129	72*	141			4	4
27	91	228	91	186*	91	213	78*	200			4	4
June 6	93	241	89	215	88	230	87	244			4	4
1942	Not treated		Spergon		Semesan Jr.		Fermate					
May 11	44	163	48	162	43	124	47	177			6	6
21	73	143	78	131	77	125*	77	127*			6	6
June 13	87	.....	88	.....	85	.....	90	.....			3	...
29	69	.....	77	.....	67	.....	73	.....			3	...
July 6	66	.....	72	.....	67	.....	73	.....			3	...
7	68	.....	65	.....	72	.....	70	.....			3	...
1943	Not treated		Spergon		Semesan Jr.		Arasan					
May 18	62	.....	75*	.....	64	.....	.....	.....			6	6
25	79	181	82	185	79	166	.....	.....			6	6
June 4	86	175	89	185	88	201	.....	.....			6	6
11	84	105	88	129	87	96	87	113			6	6

NOTE: Figures marked * are significant as compared with untreated check.

undoubtedly the results were influenced by local variations in temperature and rainfall. Spergon improved the stand in three of eight plantings. Red Cuprocide was beneficial in two cases and detrimental in two others. Yellow Cuprocide reduced the stand in six plantings and the figures are significant.

In 1940 the first planting at the Research Farm was in soil with a high moisture content and rain fell shortly after planting. Both Spergon and Semesan Jr. failed to give any significant difference in stand. At the time of the second planting on May 29 soil moisture was low and no rain fell for nearly two weeks after planting. Under these conditions all of the materials used significantly increased the stand.

Tests made on farms of growers in 1940 were not too favorable for seed treatment. Spergon increased the stand in nine plantings and actually decreased it significantly in three of twenty-one tests. In the same number of tests Semesan Jr. increased the stand in four cases and decreased it in three. In most cases where the stand was increased by seed treatment it occurred in the earliest plantings made in a locality.

In 1941 the rainfall during April was very light and on May 6, when the first planting was made at the Research Farm, the soil was very dry. Rain fell two days later with the result that the stand from untreated seed was unusually good for so early a planting. The stand was significantly improved, however, where the seed was treated with Spergon and Semesan Jr. At the time the second planting was made soil-moisture conditions were more favorable for germination and Semesan Jr. was the only material used which improved stand.

Tests with growers in 1941 were confined to the use of Spergon. All of these plantings were made prior to June 1 and seed treatment increased stands significantly in four of the earliest plantings.

No improvement resulted from seed treatment in 1942. Low temperature and high soil-moisture content resulted in a poor stand in the planting made on May 11. Seed treatment did not improve the stand and it was apparent that under such conditions none of the materials used gave sufficient protection to be of any value.

Seed treatment with Spergon was of value in increasing the stand in the first planting made in 1943. There was ample soil moisture at planting time and the temperature was sufficiently high for good germination.

Yield records were not taken on all seed treatment tests but where they were taken (Tables 1 and 2) the figures show that in very few cases was the yield influenced by differences in stand due to seed treatment. At the Research Farm during the extremely dry season of 1939 the differences in stand were sufficiently large to be reflected in increased yields.

Special mention should here be made of the copper compounds used. Red Cuprocide has some value as a seed protectant for Lima beans under certain conditions but unfortunately causes a hardening of the seed coat and stunting of the seedlings which more than offsets its value as a seed

TABLE 2.—*The percentage stand and the yield in bushels an acre of Lima beans after seed treatment, on farms of growers on Long Island*

Date planted	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Number of replicates	
1939	Not treated		Spergon		Red Cuprocide		Yellow Cuprocide		Stand	Yield
May 9	43	173	47*	178	44	148*	37*	139*	8	4
9	39	180	47*	170	47*	129*	44	180	8	4
27	45	149	49	149	47	182*	36*	134	8	4
June 3	68	162	67	153	57*	163	55*	147	8	4
5	38	129	45*	154	48*	150	23*	68*	8	4
12	95	237	95	234	94	255	90*	231	3	3
19	49	106	51	86*	45*	103	44*	77*	8	4
20	47	23	51	49	50	28	49	.....	8	4
1940	Not treated		Spergon		Semesan Jr.					
May 10	69	139	78*	173	67	180			8	4
10	91	185	93	142*	93	174			5	4
10	51	.....	60*	.....	66*	.....			10	...
14	63	123	67	154	64	138			10	4
15	65	.....	69	.....	71	.....			8	4
18	27	.....	30	.....	36*	.....			8	...
18	92	137	80*	178	92	140			8	4
20	80	113	68*	154	69*	170			8	4
30	85	.....	75	.....	85	.....			4	...
30	84	.....	80*	.....	79*	.....			8	...
30	67	.....	81*	.....	60	.....			4	...
June 4	77	.....	82*	.....	77	.....			8	...
4	67	.....	73*	.....	73*	.....			10	...
7	58	.....	63*	.....	57	.....			4	...
12	94	.....	93	.....	96	.....			5	...
18	62	.....	69*	.....	68	.....			4	...
20	56	37	67*	39	61	36			10	4
20	51	161	54	202*	55	178*			8	4
20	48	.....	45	.....	43*	.....			8	...
28	73	.....	.....	.....	79*	.....			8	...
29	75	.....	79*	.....	.....	.....			8	...
July 2	78	.....	.....	.....	78	.....			6	...
3	76	.....	76	.....	.....	.....			6	...

TABLE 2.—(*Continued*)

Date planted	Stand %	Yield *Bu.*	Stand %	Yield *Bu.*	Number of replicates	
1941	Not treated		Spergon		Stand	Yield
					10	...
April 30	33	.....	63*	.....	20	10
May 12	78	191	84*	182	12	8
12	81	121	90*	107	10	6
13	93	134	98	153	20	10
16	87	267	96*	280	8	8
19	95	204	86	218	20	10
19	83	193	79	185	12	10
27	94	201	96	171*		

NOTE: Figures marked * are significant as compared with untreated check.

protectant. This hardening and stunting effect is even more marked with both Yellow Cuprocide and copper oxychloride sulfate.

SUMMARY AND CONCLUSIONS

Seed-treatment experiments with Lima beans were carried on at the Long Island Vegetable Research Farm for five years. For three years during this period extensive tests were conducted on farms of growers. Under conditions of low soil moisture and low rainfall following planting the stand was improved regardless of temperature. Under conditions of high soil moisture and low temperature none of the materials used gave sufficient protection to be of commercial value. Except in an unusually dry season the increase in stand obtained by seed treatment was not sufficient to influence the yield.

Treatment with copper compounds hardened the seed coat and stunted the plants.

From the data presented it is concluded that on Long Island seed treatment of Lima beans has some value as a seed protectant and may be expected to improve the stand under conditions of low soil moisture at planting time followed by a period of low rainfall. The stand in plantings made in early May, when the temperature is normally low, will usually be increased as a result of seed treatment, provided the rainfall is not too heavy during the pre-emergence period.

In general, most of the materials used in these tests improved stand under conditions favorable to results from seed treatment although in some cases the number of tests was limited and the results in such cases can only be considered as indicative. Of the materials used, Spergon showed the most promise as a seed protectant for Lima beans.

On the basis of these experiments seed treatment of Lima beans can not be recommended to Long Island growers as a profitable practice.

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EFFECT OF SEVERAL SEED PROTECTANTS ON GERMINATION AND STANDS OF VARIOUS FORAGE LEGUMES[1]

J. Lewis Allison and J. H. Torrie[2]

(Accepted for publication May 10, 1944)

During the last few years recommendations have been made for the general use of seed protectants. However, little experimental evidence has been presented to date with respect to the possible effect of protectants on seed germination and subsequent stands of small seeded forage legumes. Chilton and Garber[3] summarized the literature through 1940 and presented data on the effect of seed treatment on stands of some forage legumes at State College, Pennsylvania. In greenhouse tests they obtained significant increases in stand with certain fungicides, using certain species of legumes. Other species did not respond to the various fungicides tried, and seeds of some species were injured. Kreitlow[4] reported increases in stands of alfalfa and red clover in field tests in Pennsylvania when the seed had been treated with certain fungicides. This paper presents the results from experiments at Madison, Wisconsin, in the greenhouse and the field during 1942–43.

MATERIALS AND METHODS

Medicago sativa (alfalfa), *Melilotus alba* (white-flowered sweet clover), *Trifolium pratense* (medium red clover), *Trifolium hybridum* (alsike clover), *Trifolium fragiferum* (strawberry clover) and *Trifolium repens* (ladino clover) were selected from the small-seeded legumes of commercial importance. The protectants tested were 5 per cent ethyl mercury phosphate (New Improved Ceresan), tetrachloro-parabenzoquinone (Spergon), and 50 per cent tetra-methyl thiuramdisulfide (Arasan). These dusts represent an organic mercuric and two organic, nonmercuric compounds.

A standard germination test was made on the seeds of all species tested. A 10-gram sample of each species was weighed, treated by adding an excess of protectant, shaken well, screened and re-weighed. The weight dosage was the difference in weight of the untreated and treated lots expressed in percentage of untreated seed. One hundred seeds of each treated lot and one untreated check lot of each species were plated on nutrient agar immediately following treating and again after storage for 30 days in corked glass vials.

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Forage Crops and Diseases; Bureau of Plant Industry, Agricultural Research Administration, U. S. Dept. of Agriculture. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Associate Pathologist, Division of Forage Crops and Diseases, and Assistant Professor of Plant Pathology; and Assistant Professor of Agronomy, respectively.

[3] Chilton, S. J. P., and R. J. Garber. Effect of seed treatment on stands of some forage legumes. Jour. Amer. Soc. Agron. 33: 75–83. 1941.

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In the greenhouse two different types of soil were used. Compost of pH 7.1 known to be infested with *Pythium, Rhizoctonia* and *Fusarium* spp., which cause damping-off of legume seedlings, and woods-humus of pH 6.4 which was free of damping-off organisms. The design used was a split-plot with 3 replications. The replicates were divided into blocks of 4 rows each. A block consisted of the 3 treatments and an untreated check of each species. The treatments were randomized within replicates. The rows were 20 inches long and 2 inches apart. The seed was planted immediately following treating at a uniform depth of one-half inch at the rate of 50 seeds per row. The temperature ranged between 60° and 70° F. Water was applied as a fine spray often enough to keep the surface of the soil moist.

A field experiment of the same design but on a larger scale was planted in early May, 1943, on land on which forage legumes had been in the rotation for many years. The rows, of 200 seeds, were 4 feet long and 6 inches apart. No attempt was made to alter environmental conditions.

Plant emergence counts were made on alternate days until maximum emergence for each species was determined. Final stand counts were made after all post-emergence damping-off had ceased, usually six days after total emergence. Post-emergence damping-off is defined as the death of fully emerged seedlings prior to the formation of the first unifoliate leaf and pre-emergence damping-off as the death of germinating seedlings prior to emergence. All seedlings were removed from the soil and examined for nodule development 5 weeks after planting.

EXPERIMENTAL RESULTS

Ten-gram seed lots of each species retained an equivalent amount, approximately one per cent by weight, of each of the three fungicides.

No injurious effect on germination was apparent when the treated seed of any species was compared with untreated seed in the agar plate test. However, the germination of ladino and strawberry clovers was retarded but not reduced in total when treated with Spergon. The results were the same when this test was repeated after storing seed samples for 30 days.



Table 1 summarizes the effects of the seed protectants on the total emergence and final stand of the legumes when grown in compost in the greenhouse. The differences in the values for total emergence and final stand give a measure of post-emergence damping-off. Table 2 summarizes the results obtained when the legumes were grown in woods-humus in the greenhouse. Table 3 summarizes the results of the field experiment. As there was no post-emergence damping-off with any species in woods-humus or in the field, total emergence and final stand values are the same.

The effects of the protectants were pronounced for all species in compost. All of the protectants resulted in significant increases in the total emergence of alfalfa and alsike clover. Only New Improved Ceresan significantly increased the total emergence of sweet clover. The total emergence of red clover was significantly increased by New Improved Ceresan and Arasan.

TABLE 1.—*Effects of three seed protectants on total emergence and final stand of six forage legumes grown in compost, infested with damping-off organisms, in the greenhouse, at Madison, Wisconsin*

Legumes	Germination	Total emergence,[a] number of plants[b]				Final stand,[a] number of plants[c]			
		Check	New Improved Ceresan	Arasan	Spergon	Check	New Improved Ceresan	Arasan	Spergon
	%								
Medicago sativa	75	309	424**	369**	387**	191	310**	264**	251**
Melilotus alba	60	241	287*	279	263	188	228	211	218
Trifolium pratense	60	358	434**	402*	358	315	394**	362*	324
Trifolium hybridum	60	180	278**	253**	232*	149	241**	220**	195*
Trifolium fragiferum	65	338	329	362	205**[d]	324	317	334	184**
Trifolium repens (ladino)	55	194	170	180	67**[d]	160	155	169	60**

[a] Four experiments, 12 replications.

[b] M.S.D. 1% = 56; 5% = 42. Figures significant at the 1% level are marked with a double asterisk; those significant at the 5% level, with a single asterisk.

[c] M.S.D. 1% = 57; 5% = 43. Figures significant at the 1% level are marked with a double asterisk; those significant at the 5% level, with a single asterisk.

[d] Emergence retarded.

TABLE 2.—*Effects of three seed protectants on total emergence and final stand of six forage legumes grown in woods-humus in the greenhouse, at Madison, Wisconsin*

Legumes	Germination	Total emergence and final stand,[a] number of plants[b]			
		Check	New Improved Ceresan	Arasan	Spergon
	%				
Medicago sativa	75	109	112	119	108
Melilotus alba	60	92	113**	120**	104
Trifolium pratense	60	92	92	86	96
Trifolium hybridum	60	91	78	86	77
Trifolium fragiferum	65	99	86	87	87[c]
Trifolium repens (ladino)	55	82	90	100*	97*[c]

[a] One experiment, 3 replications.
[b] M.S.D. 1% = 19; 5% = 14. Figures significant at the 1% level are marked with a double asterisk; those significant at the 5% level, with a single asterisk.
[c] Emergence retarded.

Spergon delayed emergence and significantly decreased the total emergence of strawberry and ladino clovers. None of the protectants had any effect in controlling post-emergence damping-off of any species. The effect of the fungicides on total emergence and final stand remained approximately the same for all species.

The effects of the fungicides were not pronounced for all species in woods-humus. None of the protectants had any effect on alfalfa, red clover and alsike clover. New Improved Ceresan and Arasan significantly increased the total emergence and final stand of sweet clover. Spergon retarded emergence but did not significantly decrease the total emergence or final stand of strawberry clover. Arasan and Spergon significantly increased the total emergence and final stand of ladino clover, although use of Spergon resulted in retarded emergence.

TABLE 3.—*Effects of three seed protectants on total emergence and final stand of six forage legumes in a field experiment at Madison, Wisconsin*

Legumes	Germination	Total emergence and final stand[a], number of plants			
		Check	New Improved Ceresan	Arasan	Spergon
	%				
Medicago sativa	75	354	309	325	353
Melilotus alba	60	251	219	194	224
Trifolium pratense	60	195	252	254	242
Trifolium hybridum	60	230	231	217	241
Trifolium fragiferum	65	293	256	238	252[b]
Trifolium repens (ladino)	55	318	286	259	285[b]

M.S.D. 1% = 85; 5% = 64.
[a] One experiment, 3 replications.
[b] Emergence retarded.

In the field none of the fungicides had any statistically significant effect on the total emergence or final stand of any species. Spergon, however, retarded the emergence of strawberry and ladino clovers.

Five weeks after the date of planting, the seedlings of all species in each experiment were removed from the soil and examined for nodule formation. When compared with the untreated checks, nodule development was normal for all species regardless of treatment.

DISCUSSION AND SUMMARY

The effects of three seed protectants on germination and stands of six forage legumes were studied in laboratory, greenhouse, and field experiments. Approximately one per cent by weight of each fungicide adhered to the seed of each legume. This is a heavy dosage when compared with the recommended rates of application for cereal and vegetable crop seeds. However, laboratory tests demonstrated that none of the protectants had an apparent injurious effect on germination. One fungicide, Spergon, retarded but did not reduce the germination of strawberry and ladino clovers but had no such effect on alsike clover, medium red clover, alfalfa or white-flowered sweet clover. Retardation was further accentuated when strawberry and ladino clovers were planted in different types of soil. In humus soil in the greenhouse and in the field where total emergence and final stand remained the same because no post-emergence damping-off occurred, strawberry and ladino clovers were retarded in emergence but not reduced in total emergence. However, when planted in compost known to be infested with damping-off organisms, strawberry and ladino clovers were retarded in emergence and very significantly reduced in total emergence, indicating that the protectant so inhibited germination that necessary damping-off organisms had added opportunity to cause pre-emergence damping-off or rotting of the seedlings.

In compost infested with damping-off organisms certain species were protected against pre-emergence damping-off by certain of the fungicides but none of them had any effect in controlling post-emergence damping-off with any species.

In humus soil only white-flowered sweet clover and ladino clover were benefited by any of the fungicides, and as there was no damping-off the results are interpreted as being due to a stimulatory effect of the protectants on seed germination of the two species.

In the field none of the fungicides was found to be significantly beneficial with any legume treated.

From the results obtained in all experiments it was apparent that none of the fungicides used inhibited nodule formation. Studies are in progress on the effects of adding nodule bacteria to seed treated with fungicides.

The relative value of seed treatment as a means of increasing germination and bettering stands of the small seeded forage legumes can be determined only by making many experiments in numerous localities. The ulti-

mate proof of the value of fungicides for these legumes would be the general adoption of seed treatment based upon sound experimental evidence. There would seem to be little evidence from these experiments to recommend such general adoption in Wisconsin.

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CROP ROTATION AND SOIL DRAINAGE EFFECTS ON SUGAR BEET TIP ROT AND SUSCEPTIBILITY OF OTHER CROPS TO APHANOMYCES COCHLIOIDES[1]

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The pathogenicity of *Aphanomyces cochlioides* Drechsler to tap roots of sugar beets has been reported.[3] The abundance of tip rot in some sugar beet fields and its scarcity or near absence in others in Hancock County, Iowa, in 1937, 1938, and 1939 led to an examination of the crop sequence records of such fields and to a determination of susceptibility to *A. cochlioides* of other crops grown in rotations with beets. The presence or absence of severe tip rot was also found to be associated with the locations of tile drains.

CROPS RESISTANT TO APHANOMYCES COCHLIOIDES

Crops commonly grown in rotation with sugar beets in northern Iowa are corn, oats, soybeans, alfalfa, and sweet clover. Seedlings of these crops, together with sugar beets, were exposed to *A. cochlioides* in the following manner. Soil in 36 glazed one-gallon crocks was steamed at 20 pounds' pressure for one hour. Ten-day-old cultures of two isolates of *A. cochlioides* growing on corn-meal agar were cut into centimeter squares and uniformly distributed on a plane about two inches below the soil surface in 24 crocks, at the rate of one Petri dish culture per crock. Plain corn-meal agar was likewise placed in 12 crocks. Seeds of corn (7), oats (21), soybeans (14), and sugar beets (14 clusters) were distributed uniformly on a plane one-half inch deep. There were six crock cultures for each crop, two noninfested (corn-meal agar only) checks and four cultures infested with *A. cochlioides*, two by each of two isolates. Seedling stands were recorded 12 and 17 days after planting. Final stand, length and weight of tops and roots, and presence of lesions on the roots were recorded 27 days after planting.

In the four infested crock cultures planted with sugar beets, only two seedlings remained after 27 days, and these were diseased. *Aphanomyces cochlioides* was isolated from four seedlings that damped off. In the two noninfested crock cultures, 32 and 23 healthy beet seedlings remained. The stands of the other five crops were uniformly good, and no variation in length or weight of tops and roots occurred. Numerous lesions were present on the roots of corn and soybeans grown in infested and in noninfested soils, but attempts to isolate *A. cochlioides* from these lesions failed. A repetition of this experiment yielded essentially the same results.

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[2] Plant Pathologist, South Dakota Agricultural Experiment Station, formerly research assistant, Botany and Plant Pathology Section, Iowa Agricultural Experiment Station. The author is indebted to Dr. I. E. Melhus for aid and suggestions during the course of investigations and preparation of the manuscript.

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TABLE 1.—*Crop sequences of fields in northern Iowa with different amounts of sugar beet tip rot in 1937, 1938, and 1939*

Fields 1, 2 (Nor. Iowa Exp. Assoc., Kanawha)			
	1		2
1930	Beets	1930	Beets
1931	Small grain	1931	Beets
1932	Beets	1932	Corn
1933	Corn	1933	Oats seeded to alfalfa
1934	Barley	1934	Alfalfa
1935	Beets (poor crop)	1935	Alfalfa
1936	Corn	1936	Beets (normal crop)
1937	Beets	1937	Corn
		1938	Beets
Tip rot	Crop failure because of tip rot	Tip rot	Crop failure because of tip rot

Field 3 (N. Christians, Kanawha)		
	A	B
	No beets prior to 1934	
1934	Oats	Beets
1935	Alfalfa	Corn
1936	Alfalfa	Beets
1937	Alfalfa	Corn
1938	Beets	Beets
Tip rot	None	90 pct.

Field 4 (M. Hill, Clarion)			
	A	B	C
1932	Beets	Beets	Beets
1933	Corn	Corn	Corn
1934	Oats	Oats	Oats
1935	Sweet clover	Sweet clover	Alfalfa
1936	Corn	Beets	Alfalfa
1937	Corn	Corn	Alfalfa
1938	Oats	Oats	Alfalfa
1939	Beets	Beets	Beets
Tip rot	None	50 pct.	None

Field 5 (F. L. Bubeck, Clarion)		
	A	B
1932	Alfalfa	No beets
1933	Corn	No beets
1934	Corn	Corn
1935	Oats	Beets
1936	Beets	Corn
1937	Corn	Corn
1938	Beets	Beets
Tip rot	60 pct.	20 pct.

TABLE 1—(*Continued*)

Fields 6, 7 (A. Christians, Kanawha)		
	6	7
1932	Pasture	No beets
1933	Corn	No beets
1934	Oats	Beets
1935	Beets	Corn
1936	Corn	Oats
1937	Oats	Sweet clover, alsike, timothy
1938	Beets	Beets
Tip rot	70 pct.	10 pct.

Field 11 (D. Waggoner, Kanawha)	
1933	Beets
1934	Oats
1935	Corn
1936	Oats
1937	Sweet clover
1938	Beets
Tip rot	95 pct.

Field 13 (Nor. Iowa Exp. Assoc., Kanawha)	
1930	Beets (seedlings killed)
1931	Corn
1932	Beets (failure: tip rot)
1933	Corn
1934	Flax
1935	Alfalfa
1936	Alfalfa
1937	Alfalfa
1938	Alfalfa
1939	Beets
Tip rot	None

Fields 8, 9, 10 (N. E. Schroeder, Clarion)			
	8	9	10
1933	Beets	No beets prior to 1937	
1934	Oats		
1935	Sweet clover		
1936	Beets		
1937	Corn	Sweet clover	Sweet clover,
1938	Beets	Beets	Beets
Tip rot	50 pct.	None	None

Field 12 (H. Christians, Kanawha)	
	No beets prior to 1935
1935	Beets
1936	Corn
1937	Oats
1938	Sweet clover
1939	Beets
Tip rot	None

Barley, red clover, flax, sugar beets, pigweed (*Amaranthus retroflexus* L.), and lamb's quarters (*Chenopodium album* L.), the latter two close relatives of the sugar beet, were exposed to *A. cochlioides* in two experiments identical to the one described except that pots were used instead of crocks. Barley and red clover were not parasitized by *A. cochlioides*. In the first experiment flax germination apparently was interrupted by *A. cochlioides*, as the stand was less in infested soil than in noninfested soil; but this difference did not occur in the second trial. All seedlings of sugar beet, pigweed, and lamb's quarters grown in infested soil were infected, and *A. cochlioides* was isolated from one or more seedlings of each. All seedlings grown in noninfested soil were healthy. The susceptibility of pigweed and lamb's quarters to *A. cochlioides* and their general distribution in northern Iowa may account for the presence of this organism in northern Iowa soils. In many experiments with steamed soil, there has been no evidence that *A. cochlioides* is seed-borne.

OCCURRENCE OF TIP ROT IN FIELDS WITH KNOWN CROP SEQUENCES

The crop sequences of two adjoining fields (1 and 2) on the Northern Iowa Experimental Association Farm at Kanawha that failed because of tip rot in 1937 and 1938 and the sequences of various commercial fields showing severe, slight, or no damage from tip rot in 1938 or 1939 are recorded in table 1.

In the commercial fields large samples could not be removed for loss estimates. Usually two people visited the fields and agreed upon an estimate of the damage after examining a few beets from each field under observation. Although these estimates may not be accurate it can be said that clear-cut differences in the amount of tip rot existed and that they were in the direction of and in proportion to the estimates recorded.

In fields 3, 4, and 5, the various portions with different crop sequences were distinguished by different amounts of tip rot (Fig. 1). Such differences were apparent across the entire field and virtually to the row.

The most striking case observed was field 3. Part B was a virtual failure because of tip rot. It was in beets for the third time in five years. The remainder of the field (A) was in beets for the first time and had been preceded by alfalfa; it was an exceptionally good crop and free of tip rot. The two portions of this field are shown in figure 1. Field 4 was of special interest because it was in three parts. Part B was in beets the third time in eight years, with only two crops, corn and oats, intervening between the 1936 and 1939 beet crops. This part of the field suffered a 50 per cent loss from tip rot. Parts A and C, without the 1936 beet crop, had no noticeable amount of tip rot. In field 5, part A had only one corn crop between beet crops and an estimated 60 per cent of tip rot; part B had two intervening corn crops, with an estimated 20 per cent of tip rot.

The principal difference between fields 6 and 7 was the extra crop year (sweet clover, alsike, timothy) between sugar beet crops in field 7, with

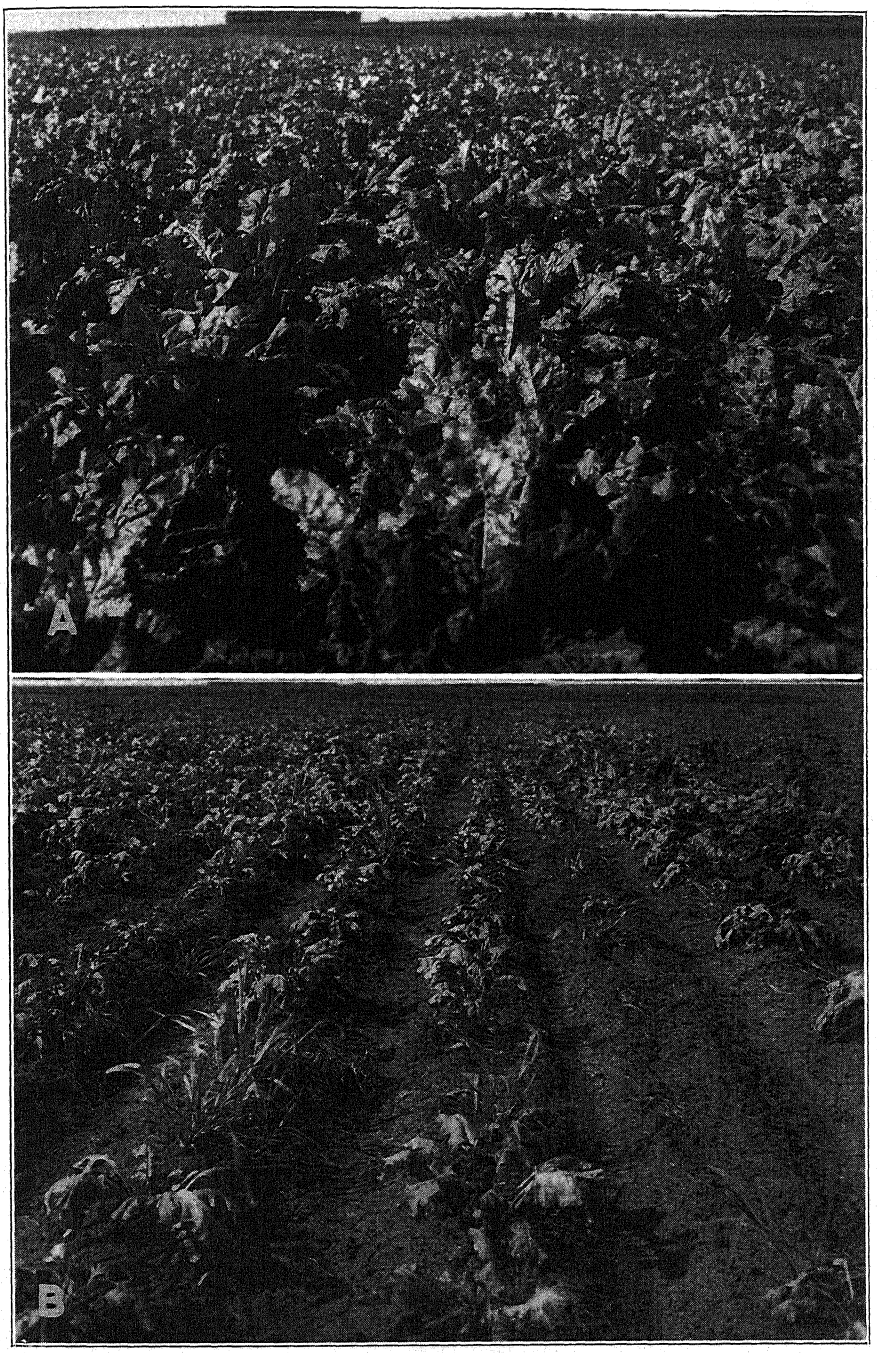


FIG. 1. A. Tip-rot-free portion of field 3, which was in beets for the first time and had previously been in alfalfa. B. Beet failure because of tip rot in another portion of field 3. This was the third crop of beets in five years, with two intervening crops of corn.

also much less tip rot. Field 8 had three crops of sugar beets in six years, with only one intervening corn crop between the last two beet crops. The second (1936) beet crop, according to the owner, was a normal one. The third (1938) beet crop suffered an estimated 50 per cent loss from tip rot. Fields 9 and 10 on the same farm were producing their first beet crop and had no tip rot. Field 11 is recorded because it seems to be a glaring anomaly. There were four intervening crops between the 1933 and 1938 beet crops, yet almost a complete loss from tip rot occurred. This field, however, was the only bad case of tip rot observed on other than Clarion or Webster soils; it was of the Fargo series. Field 12, in sharp contrast, had no tip rot, with only three crops (corn, oats, sweet clover) intervening between beet crops.

In five other fields, two in 1938 and three in 1939, sugar beets were growing on a piece of land for the first time. All five fields were free of tip rot.

In three cases (Fields 3B, 4B, 8), sugar beets occurred on the land three times in eight years or less. In all three fields, the third crop was severely tip rotted. In three cases (Fields 3A, 4C, 13), sugar beets followed three or four years of alfalfa; in none of the three was tip rot found. In one of these (Field 13), at least one previous beet crop had been a failure because of tip rot.

These general statements are ventured regarding the appearance of tip rot under various crop sequences: (1) Tip rot did not occur in the first crop of beets on a given piece of land. (2) The third crop of beets in 8 years or less (Fields 3B, 4B, 8) was severely tip rotted. (3) There was no indication of severe tip rot after alfalfa (Fields 3A, 4C, 13), even in one field where tip rot had at one time previously occurred (Field 13).

It seems therefore that a four-year rotation should prove adequate for the most part to prevent severe losses from tip rot. In a field where tip rot is serious, however, an interval of six or more years may be necessary to "clean up" the land for the next beet crop, including perhaps a three- or four-year period devoted to alfalfa, as in field 13.

TIP ROT IN RELATION TO TILE DRAINS

An unusual distribution of tip rot in field 2, 1938, was determined to be coincident with the distribution of tile drains. Three areas, 45×375, 33×375 and 27×375 ft, had, by actual count, a rather uniform 75 per cent stand of healthy beets on August 19. In 10-ft. borders around these areas the stand of healthy beets fell off sharply and by actual count was 27.3 per cent. In the rest of the field, the stand of healthy beets was 10.3 per cent. Nearly all the loss of stand was the result of tip rot.

When the presence of tile drains in the center of the relatively tip-rot-free areas was postulated, six holes were dug to determine the presence or absence of tiles. In four locations at the exact centers of the three relatively tip-rot-free areas, tiles were found at a depth of about 5 ft. Tiles were absent in two other locations, one beyond the widest tip-rot-free area and

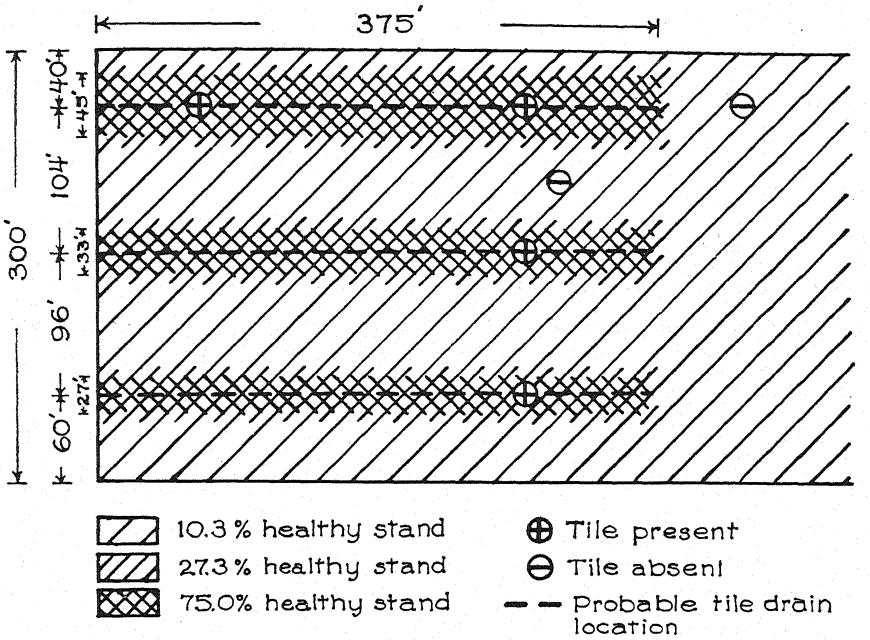


FIG. 2. Locations of tile drains in field with various amounts of tip rot.

exactly in line with the tile drain already located, the other exactly between this tile and the tile located in the adjacent tip-rot-free area.

The positions of the relatively tip-rot-free areas, the postulated locations of tile drains, and the points where tiles were or were not found are shown in figure 2.

Moisture determinations in duplicate were made of soil samples taken from three depths directly over the two south tile drains, between these two, and north of the central line. The results are tabulated in table 2.

The lower moisture content over, as compared with the area between, the tiles is evident. The difference probably is not significant in itself, but these determinations were made when the soil, though not dry, was definitely not water-logged. The farm foreman, who attended to planting and cultivation

TABLE 2.—*Moisture content of soil samples from three depths over and between tile drains, field 2, Kanawha, August, 1938*

Depth (inches)	Percentage moisture					
	Over tile drains			Between tile drains		
	Middle tile	South tile	Average	North of middle tile	South of middle tile	Average
0–6	30.1	28.9	29.5	31.8	33.4	32.6
6–12	28.1	26.5	27.3	28.4	32.5	30.4
12–18	25.5	25.2	25.3	26.0	27.4	26.7

of this field, observed that on several occasions in the spring the areas over the tile drains were dry enough to cultivate before the areas between tiles.

In two other fields near Kanawha less than the field average of tip rot occurred in the areas that the farm operators indicated were over tile drains. The presence or absence of tiles was not determined by digging.

In two greenhouse experiments, sugar beets were grown in steamed soil and in steamed soil infested with *A. cochlioides,* both subjected to limited irrigation from above in contrast to abundant irrigation from above and below. In neither experiment was there a difference in amount or degree of infection of the beets by *A. cochlioides* that could be attributed to the difference in irrigation.

The association of tile drains with less tip rot in field 2 and perhaps two other fields was so clear-cut as to allay any doubts of its existence, but the relationship between tip rot severity and excessive soil moisture, although indicated, was not definitely established.

SUMMARY

The roots of corn, oats, soybeans, alfalfa, sweet clover, barley, and red clover were not infected when grown in soil infested with *Aphanomyces cochlioides.* Germination of flax apparently was interrupted by *A. cochlioides* in one trial, but in the second experiment flax did not appear to be susceptible. The roots of sugar beets, pigweed (*Amaranthus retroflexus* L.), and lamb's quarters (*Chenopodium album* L.) were infected. The susceptibility of these two common weeds suggests the universal presence of *A. cochlioides* in northern Iowa soils.

In fields with varying amounts of sugar beet tip rot the following observations were made: (1) In no case did tip rot occur in the first crop of beets on a given field. (2) The third crop of beets in 8 years or less was severely tip-rotted. (3) There was no indication of severe sugar beet tip rot after alfalfa, even in one field where tip rot had at one time previously occurred.

Intervals of three or four years between beet crops should adequately avoid severe losses from tip rot. However, an interval of six or more years, including three or four years of alfalfa, may be desirable for a field in which sugar beet tip rot has been severe.

Tile drains were definitely associated with less than the field average of sugar beet tip rot at Kanawha in 1938. The relationship between tip rot severity and excessive soil moisture was indicated but not definitely established.

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INFECTION PHENOMENA IN TOMATO-FRUIT ROT CAUSED BY PHYTOPHTHORA CAPSICI[1]

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Recent investigations on the tomato-fruit-rot disease in Colorado caused by *Phytophthora capsici* Leonian have disclosed the advisability of using histological studies in current pathological research.[2] Initial invasion, the development of the causal agent in host tissue, and subsequent sporulation of the organism have been studied histologically. Another phase to merit attention was the investigation of an observed difference between stylar- and stem-end tissues to infection.

MATERIALS AND METHODS

Green tomato fruits of the Landreth variety were inoculated by placing drops of water containing active swarmspores on the uninjured surfaces of the fruits. The swarmspore inoculum was prepared by the soil-inoculum technique.[3] The inoculated fruits then were incubated in moist chambers at 25° C. for varying times.

After incubation periods of 2 to 72 hours, small pieces for histological studies were cut from the areas where the swarmspores had been placed. These pieces were killed and fixed in formalin-acetic-alcohol and in several of the chrom-acetic and chrom-acetic-formalin solutions described by Sass.[4] The "Craf" I and "Craf" II formulae gave the best results and consequently were used most frequently. Tertiary butyl alcohol was the best dehydrating agent when used according to Johansen's method[5] as modified by Sass.[4] Ethyl alcohol was usable, but dioxan was not satisfactory as a dehydrating agent. Sections 12 to 20 microns thick were best for these studies. Phloxine and fast green F. C. F. proved most satisfactory of the several staining combinations employed.

RESULTS

Primary Invasion and Infection. Hyphae from germinated swarmspores penetrated the cuticle of inoculated fruits in a relatively short time. After a 2- to 3-hour incubation large numbers of the spores had produced appressoria and the constricted invasion hyphae arising therefrom had extended into the cuticle (Fig. 1, B). Germinating spores frequently were scattered over the entire surface area covered by the drop of inoculum. In other in-

[1] Paper No. 184 of the Scientific Journal Series of the Colorado Agricultural Experiment Station.

[2] Cooperative work conducted with Dr. L. R. Bryant, Section of Horticulture, Colorado Agricultural Experiment Station.

[3] Kreutzer, W. A., and L. R. Bryant. A method of producing an epiphytotic of tomato-fruit rot in the field. Phytopath. 34: 845–847. 1944.

[4] Sass, John E. Elements of botanical microtechnique. 222 pp. McGraw-Hill (New York). 1940.

[5] Johansen, D. A. Dehydration and infiltration. Science 82: 253–254. 1935.

stances large clusters of spores were covering and adjacent to areas where epidermal hairs apparently had been broken off (Fig. 1, A). In some cases the constricted invasion hyphae grew directly into the epidermal cells; in other cases the penetrating hypha apparently encountered a more resistant structure in the cutinized wall and grew tangentially as far as the junction of two epidermal cells. In the latter the hypha grew radially into the

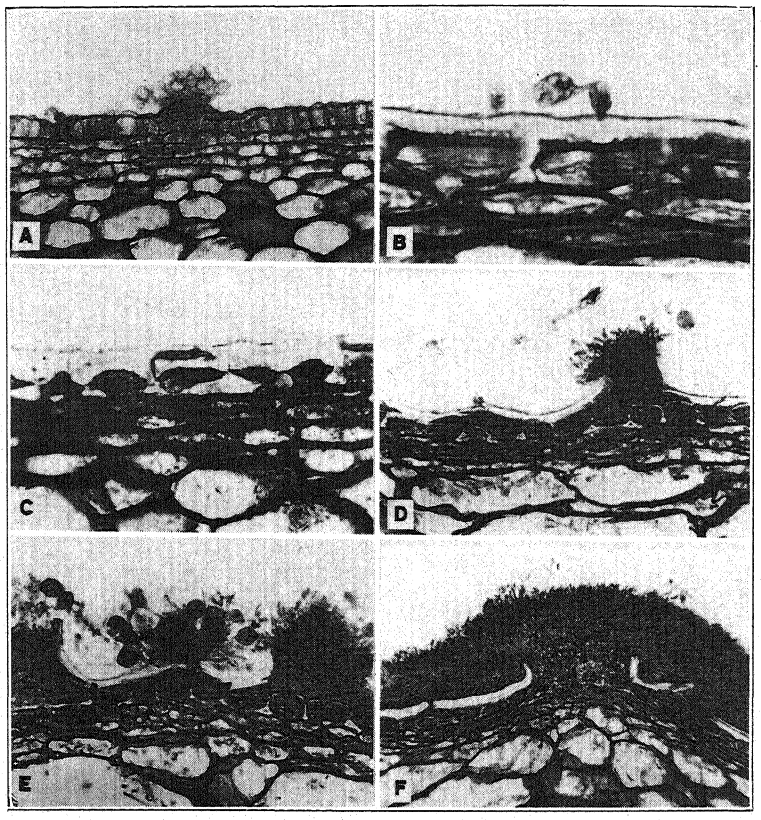


FIG. 1. Sections of tomato fruits (variety Landreth) showing invasion and fruiting of *Phytophthora capsici* Leonian. A. Swarmspores collected around a broken hair base on a green fruit, 70 min. after inoculation. B. A germinated swarmspore showing appressorium with invasion hypha entering the cuticle, 2 hr. after inoculation. C. Hypha penetrating cuticle (spore evidently removed in handling), 24 hr. after inoculation. D. Accumulated hyphae in epidermal and subepidermal cells causing rupture of the cuticle. E. Sporangia in the fungal eruption areas. F. The thick mycelial mass enclosing sporangia.

protoplast of an epidermal cell (Fig. 1, C). This condition was more frequent in tomatoes which were beginning to turn pink.

Following primary invasion, the outer tangential walls of the epidermal cells were distended by the accumulating underlying hyphae. Upon rupture of the epidermal cells and cuticle, hyphal masses emerged at points of rupture (Fig. 1, D).

Sporulation on Host Tissues. Both naturally and artificially infected fruits were examined in order to study the nature of reproduction and time required for probable sporangial formation on host tissues. Hyphal eruptions were macroscopically evident as mycelial mats of varying sizes when fruits were inoculated and held in moist chambers at 25° C. for 72 hours. Only the early stages of sporangial formation were observed in vertical sections through such areas. Fruits with the earliest indications of infection in the field had similar mycelial mats at the end of 48 or 72 hours when placed in moist chambers at 25° C. In many instances characteristic mature and immature sporangia were clearly evident in the smaller external hyphal growths (Fig. 1, E). These structures were present in the larger mycelial mats but were embedded in the hyphal matrix and consequently were not clearly visible (Fig. 1, F).

Infection Reactions in Stylar- and Stem-end Tissues. It had been observed in laboratory and field that infection of fruits occurred more readily through the stylar end than through the stem end. This difference in reaction to infection was more clearly evident in the laboratory trials because of superior controls. In general, infection occurred in 70 to 90 minutes at 25° C. in 98 per cent of the trials when swarmspores were placed on the stylar end of the tomato. In many instances where swarmspores were placed on the stem end they failed to cause typical infection areas even after 8 days incubation (Fig. 2, D and E). On the areas where the swarmspores had been placed were one to many small, sunken, rust-colored, arrested lesions without any evidence of typical watery breakdown.

In one typical trial characteristic water-soaked fruit-rot areas were evident 20 hours after inoculation as a result of all stylar-end inoculations, while there was no such breakdown from stem-end inoculations. Rust-colored arrested lesions gradually became evident on the latter inoculated fruits, and after 6 days of incubation pieces for histological examinations were taken from the affected areas (Fig. 2, E).

Except for a thicker cuticle on the epidermal cells of the stylar end, the cells of normal tissue from the two ends of the fruits were similar in structure. However, the cells of the stem-end tissue contained numerous crystals (Fig. 2, A), while no such crystals were found in tissue from the stylar end (Fig. 2, C). There were three distinct crystal types as well as certain small, non-plastid, globular masses present in the stem-end sections. Many of the crystals were six-sided, prismatic in form, and apparently of either the monoclinic or hexagonal system. Other crystals appeared cubical in cross section; many of them were elongated (Fig. 2, A) and the remainder were raphide clusters. These crystal types did not always occur in the same proportions in all fruits; sections from other tomatoes revealed a greater abundance of crystals of the raphide type.

That the crystals or some other accompanying chemical substance may have had some effect on the fungus was indicated by the fact that the hyphae which penetrated the epidermis of the stem end did not spread through the

underlying tissue as was the case when the fungus entered the stylar end. In stained sections of the arrested lesion area was an accumulation of a gum-like, reddish-brown material in the epidermal and subepidermal cells (Fig. 2, B). Often a complete breakdown of the subepidermal cells occurred in this area. Apparently the hyphal development was stopped and the characteristic watery breakdown did not result (Fig. 2, D and E).

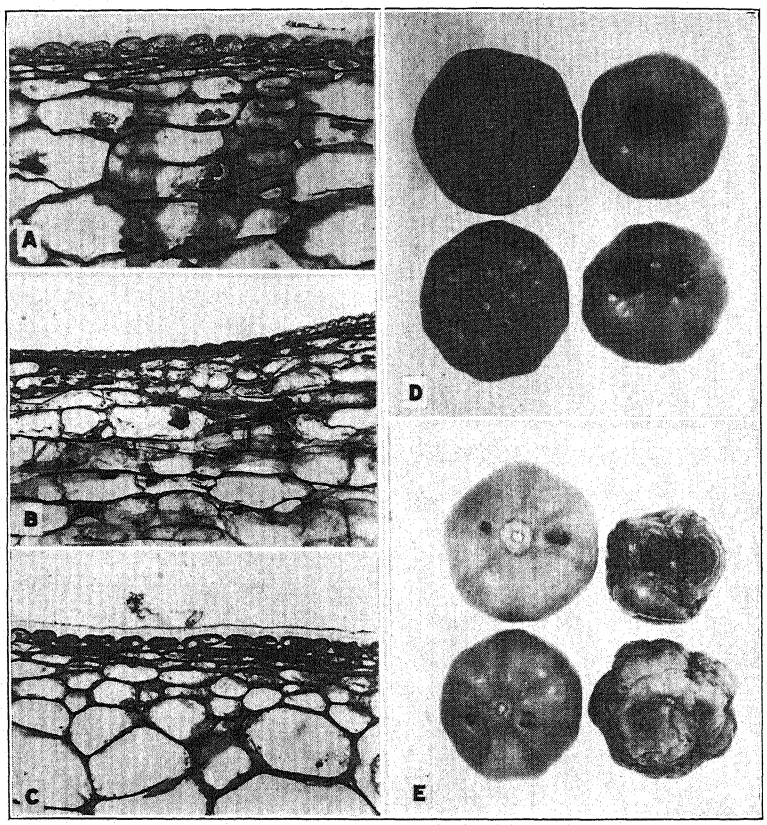


FIG. 2. Infection reactions of stylar- and stem-end tissues of tomato fruits. A. Section from stem end of Landreth tomato fruit showing presence of crystals. B. Arrested invasion in stem-end section 6 days after inoculation. Note crystals in the tissue. C. Stylar-end section showing the absence of crystals. A germinated spore is on the cuticle. D. Arrested stem-end and active stylar-end lesions in the Nebraska 642 tomato 2 days after inoculation. The variety Landreth showed the same type of reaction. E. Same fruits as in D, 8 days after inoculation.

Dufrenoy[6] observed, in cells of plants other than tomato, tannin and phenolic compounds arising as a result of the stimulus of invasion by a given pathogen. Since the bodies herein reported apparently were present in stem-end tissues of tomato fruits prior to inoculation and did not develop as a result of fungus attack, it is questionable whether they are of the same general type as those mentioned by Dufrenoy. The writers do not wish to

[6] Dufrenoy, J. Cellular immunity. Amer. Jour. Bot. 23: 70–79. 1936.

imply that the presence of these bodies in the cells of the stem-end tissues of the tomato fruits studied was directly responsible for the apparent resistance to infection. A correlation, however, existed between the presence or absence of these structures and the resistance or susceptibility of the fruit tissues tested.

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HISTO-PATHOLOGIC CHANGES IN THE PHLOEM OF AMERICAN ELM AFFECTED WITH THE VIRUS CAUSING PHLOEM NECROSIS

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INTRODUCTION

Phloem necrosis of elm was identified as a virus disease by Swingle (14) in 1938. A characteristic symptom of the disease is a yellow to brown discoloration, sometimes containing small brown to black flecks, which develops in the phloem or inner bark. In large trees, the discoloration is usually observed only in large roots and in the lower trunk. In small trees, it may occur also in the upper stem and branches. The moderately discolored tissue has a faint odor of wintergreen which cannot be detected in corresponding tissue of healthy elms. The phloem discoloration accompanied by the wintergreen odor are the only distinguishing symptoms of the disease.

As stated by Swingle (15) and as observed in inoculated 2- and 3-year-old trees cultured in soil and in a "complete" aqueous culture solution, the small fibrous roots die first. Necrosis progresses from the fibrous roots into larger roots and finally, the inner phloem in the lower portion of the stem may be killed. After the initial appearance of root symptoms, such trees usually die in two to six months. No cases of recovery have been observed. Older trees, after inoculation, may be infected for six months to one or more years before disease symptoms become apparent. Nothing is known of the duration of the "incubation period" in naturally infected trees.

The virus is readily transmitted by grafting diseased bark tissue to roots, trunk, or branches of healthy suceptible elms. Other attempts to transmit the virus by various mechanical and manual methods have failed. A natural vector of the virus has not been discovered.

Certain histo-pathological changes occur in the cells of diseased phloem tissue. This paper describes these microscopic, pathological changes in relation to the progress of the phloem necrosis from the initial infection.

REVIEW OF LITERATURE

Esau (6) summarized the anatomical changes associated with virus infections. Viruses may cause death of cells (necrosis), or an increase in size and number of cells (hypertrophy and hyperplasia), or an inhibition of growth and differentiation (hypoplasia). Two or three of these types of reactions may occur in combination. The most extensive literature on phloem degeneration pertains to the leaf-roll disease of potato reviewed by Quanjer (12) and the effects of the curly-top virus in sugar-beets and tobacco summarized by Esau (3, 4, 5, 7).

Phloem necrosis has been reported in the stripe disease of corn by Cook (1) and in the leaf-curl of raspberry by Rankin and Hockey (13). Hartzel

(8) observed hypertrophy of the phloem cells in peach trees affected by the virus causing yellows. Hyperplasia of the phloem and of the parenchyma around the phloem was observed by Magee (11) in the bunchy-top disease of banana. Lyon (10) and Kunkel (9) noted that hyperplasia and hypertrophy resulted in the formation of galls in the phloem of cane affected by the "Fiji" disease. Phloem degeneration in sugar-beets affected by curly-top is characterized by a combination of necrosis, hypertrophy and hyperplasia as noted by Esau (2, 3, 4).

EXPERIMENTAL METHODS AND RESULTS OBTAINED

To facilitate the study of root infection, inoculated 2-year-old trees were cultured with their roots suspended in a "complete," aerated, aqueous culture solution. By this method, trees could be lifted from the solution at any time and returned after examination. Inoculations were made by patch grafting diseased bark tissue to the lower portion of the stems of the healthy elms.

The first dead roots were observed 50 days following inoculation. Necrosis appeared to progress from the tips of a few rootlets. After necrosis had advanced into the larger roots, other rootlets were killed. These progressive stages of dying roots did not differ essentially from the same progressive dying observed in 2- and 3-year-old trees growing in soil where death of fibrous roots occurred one to several months after artificial inoculation.

The development and structure of the phloem tissue of the American elm has not been described previously. In the following discussion of the anatomy of the diseased tissue, reference is made to the healthy structure only where it is needed for comparison. Studies were made on the ontogeny of the root tissues of healthy American elm trees in order to be able to interpret the structure and development of comparative tissue in diseased trees.

STRUCTURE AND DEVELOPMENT OF ROOT TIPS OF DISEASED PLANTS

The general pattern of the meristematic apices of roots from diseased plants does not deviate discernibly from that of healthy roots. The arrangement of cells in the cortex and stele, and the zones of maturation in the latter, are likewise unaltered in the diseased roots. Protophloem sieve tubes are the first vascular elements that mature in the roots. In phloem tissue developing after infection, degeneration seems to follow the maturation of the primary sieve tubes. Cells immediately adjacent to these sieve tubes may undergo degeneration (Fig. 1, A and B). Usually, hypertrophy of the nuclei and cells surrounding the mature sieve tubes is the most conspicuous microscopic symptom in the primary tissues. Hypertrophied nuclei and enlarged cells are sometimes observed in the pericycle of the primary tissues. Hyperplastic cell divisions in the procambium usually follow or accompany the primary degenerative changes and continue after secondary growth

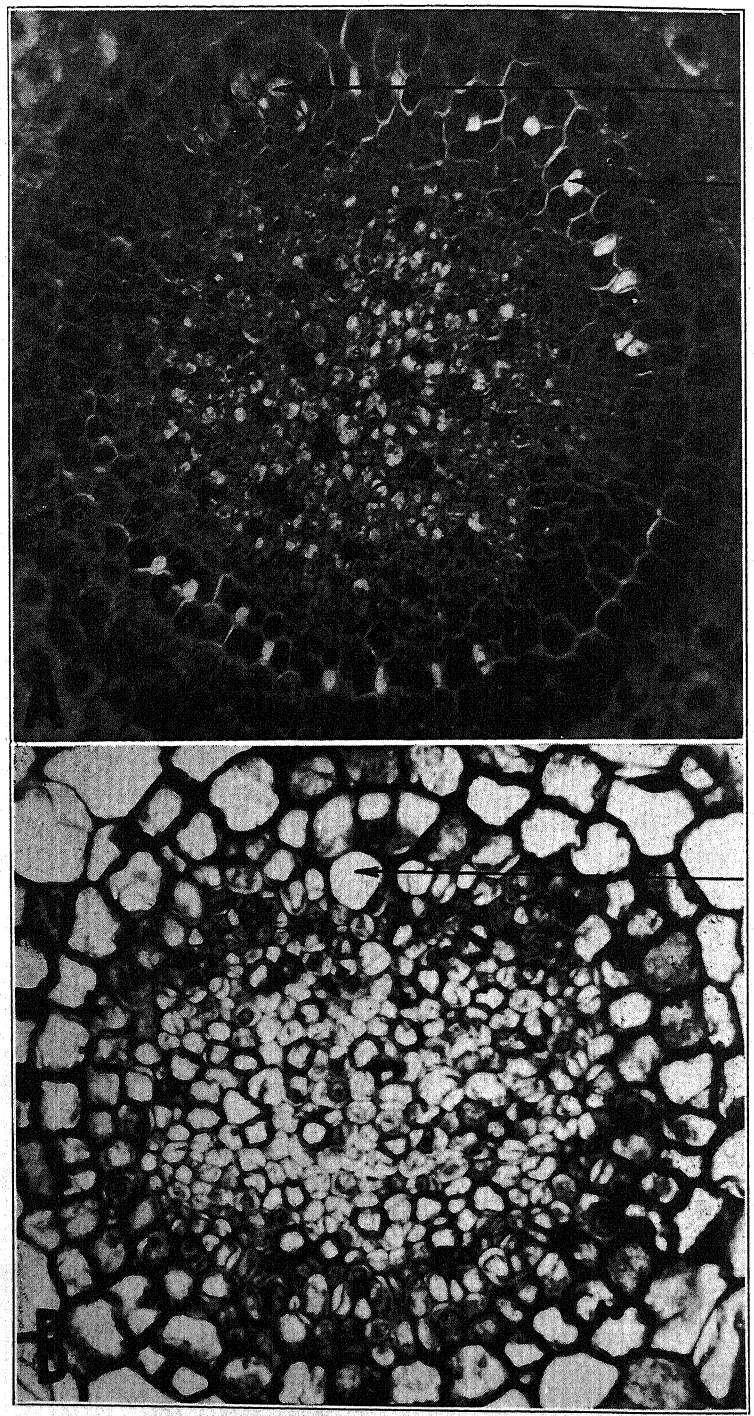


FIG. 1. Transverse sections of a root tip from a diseased American elm. The stele and part of the cortex are evident. A. Protophloem sieve tubes and hypertrophied cells adjacent to a mature sieve tube. About 700 microns from apex (×320). B. About 1200 microns from apex (×395).

begins. This symptom is conspicuous in the phloem tissue of the root or lower stem of diseased plants where the typical discoloration is apparent prior to the death of the tree (Figs. 2 and 3). Certain cells of the protophloem may undergo necrosis. Before vacuolation of cells in the regions of the primary phloem mother cells, certain of the isodiametric cells stain very darkly. These cells appear to be immature sieve-tube cells. In older roots, some of the sieve-tube cells undergo degeneration, which may indicate that the sieve tubes are primarily concerned in virus transport as well as being the seat of necrosis. These necrotic cells are usually compressed by adjacent parenchymatous cells.

STRUCTURE OF SECONDARY TISSUES IN HEALTHY AND DISEASED ROOTS

Secondary thickening of the root results from cambial activity which occurs in the zone of fundamental parenchyma lying between the central metaxylem vessels and the primary phloem. Secondary xylem consists of large vessels, smaller conductive cells, fibers and parenchyma. The parenchyma appears as rays that are from one to several cells in width, the cells being radially elongated. The large vessels form in radial rows typical of spring and summer growth, but become somewhat irregular in arrangement in the older annual rings owing to the increase in their size. The typical diarch protoxylem retained its identity in the center of the woody cylinder in roots up to 3 years old in the preparations studied.

Outside of the cambial zone, the secondary phloem has a layered appearance which results from the differentiation of zones of small, thick-walled fibers, that alternate with regions of parenchyma, sieve tubes and companion cells. The sieve tubes lose their identity in the outer phloem and only the fibers and parenchyma cells are conspicuous. Parenchymatous ray cells in the phloem are continuous with the xylem rays. The ray cells are apparently of cambial origin. As the root increases in size, the cortical cells and epidermal cells are stretched and ultimately disintegrate. Distortion of the cortical tissue in diseased material due to the virus infection has not been observed. During the secondary phases of growth, the pericycle remains active and a phellogen develops from it. The bark tissue of older roots, following the normal disintegration of the cortical tissue, consists of phloem tissue derived from the primary cambium with an outer corky periderm.

The same developmental pattern is characteristic in the diseased as in the healthy root. Xylem of diseased plants does not differ from that of healthy plants. After infection, the recently developed phloem next to the cambium zone has no regularity in cell differentiation (Fig. 2, A). Degenerative changes are more noticeable in the tissue developed subsequent to infection.

The most striking characteristic of older diseased phloem tissue of both root and stem under microscopic observation is the almost complete destruction of sieve-tube cells, with a marked increase in number and size of the parenchyma cells. Some cells divide in any plane, others enlarge without

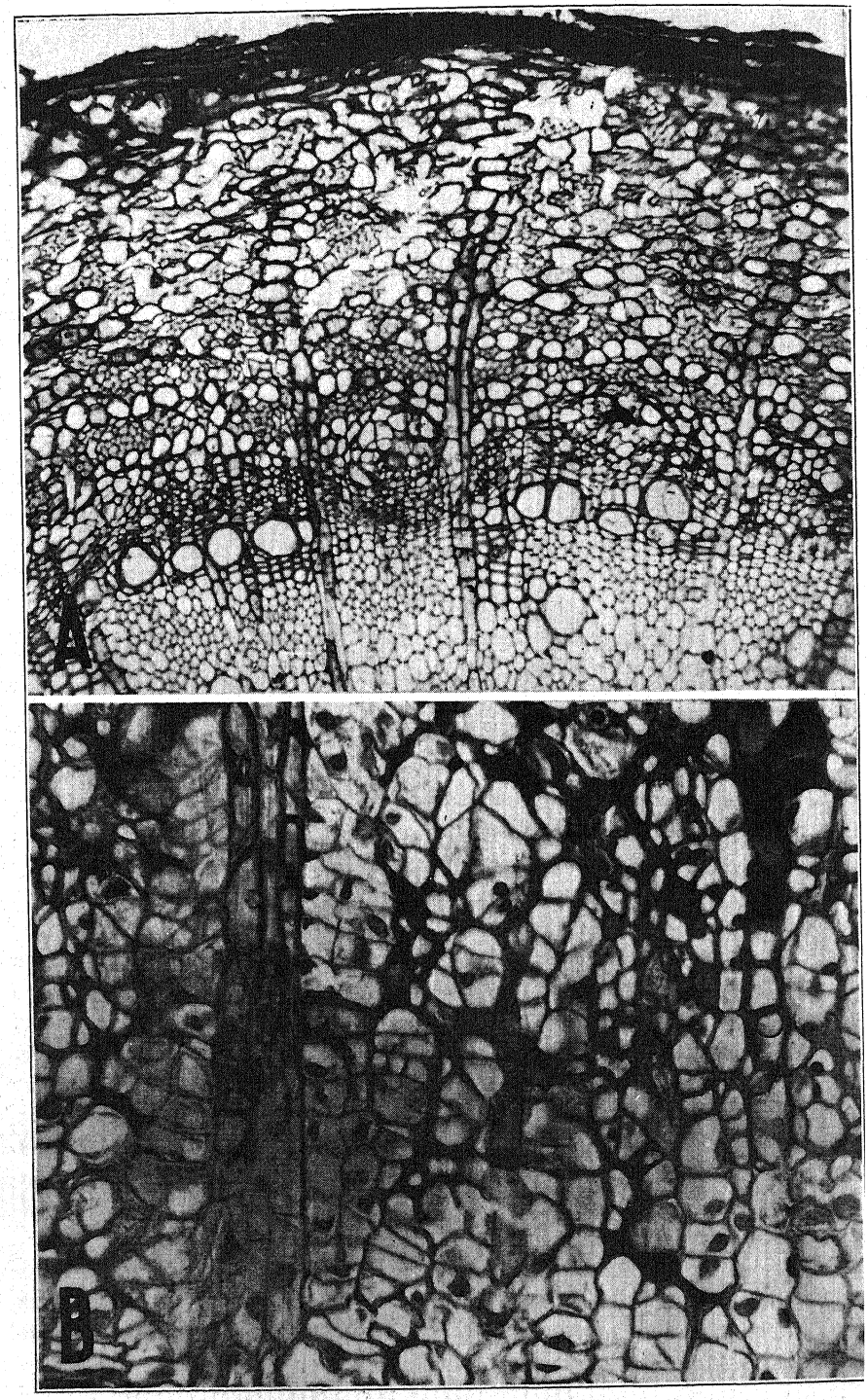


FIG. 2. A. Phloem and part of the xylem as seen in transverse sections of a diseased 3-year-old American elm root (×117). B. Necrosis, hypertrophy and hyperplasia in a transverse section of diseased phloem tissue from stem (×435).

dividing, and their contents disintegrate. In later stages, certain cells collapse, resulting in the formation of cavities which are filled with degenerate cell material and a yellowish, dark-staining, granular substance (Figs. 2 and 3).

Occasionally, the yellowish substance becomes concentrated immediately adjacent to the cell walls, resulting in irregular thickenings. Usually, it is dispersed throughout the lumina of certain phloem cells and dissolves out in fixation. Preliminary microchemical studies indicated that this material has certain properties of suberin. It seems to be anisotropic and stains yellowish-red with Sudan III.

Sieve tubes are difficult to locate in prepared sections of the hyperplastic tissue. In transverse sections, they are of about the same size as unaltered parenchyma cells, have transverse sieve plates, and are usually surrounded by abundant thin-walled parenchyma. The lateral walls of sieve-tube cells, which abut upon other sieve tubes in the secondary phloem, are frequently covered with pronounced lattices. The affected cells are crushed following enlargement and excessive divisions of the surrounding cells. The degenerated cells are destroyed and the yellowish, dark staining material may run between adjacent parenchyma cells and fuse with that of other degenerated cells (Figs. 2 and 3).

STRUCTURE OF SECONDARY TISSUE IN HEALTHY AND DISEASED STEMS

The structure of the woody cylinder of the stem has essentially the same pattern as the woody cylinder of the root. The xylem of the stem is ring porous and is transversed by numerous parenchymatous rays which have a common union surrounding the centrally located pith. Growth rings can be distinguished in the xylem owing to somewhat smaller and more compactly arranged vessels at the outer limit of each ring. The xylem tissues in diseased plants do not differ from comparable tissues in healthy plants.

Secondary phloem consists of sieve tubes, companion cells, fibers, and parenchyma. It has a banded appearance, much the same as the older phloem tissue of the roots, owing to the differentiation of tangential bands of fibers which form the outermost portion of each growth zone. The intervening bands of sieve tubes, companion cells and parenchyma may become crushed in the natural growth processes and increase in diameter of the stem. A phellogen layer, from which a periderm develops, differentiates in the cortex beneath the epidermis. During successive growth periods, the phellogen differentiates deeper in the cortex and finally in older stems the cortex is sloughed off and the bark consists entirely of phloem tissue with a corky periderm and has much the same structure as the bark of older roots. As in the roots, sieve tubes are difficult to distinguish in the older phloem tissue of stems.

The structure of diseased phloem in the stem is similar to that in diseased roots, except that hyperplasia seems to be more noticeable (Figs. 2, B, and 3). This may be explained from the fact that roots die first, while the

degeneration in the phloem of the stem may be carried over a longer period of time.

In fresh sections of bark tissue cut from a diseased tree, the yellow to brown discoloration, associated with a faint wintergreen odor, distinctly marks the boundary of the current phloem development. Observed macroscopically, it does not appear to extend into the older phloem tissue, and has never been observed in the xylem. Occasionally a yellow to brown dark-staining amorphous material is present in diseased cells. This material may be finely granular or consist of irregular globular masses. It is not known what relation this material has to the presence of virus in the discolored

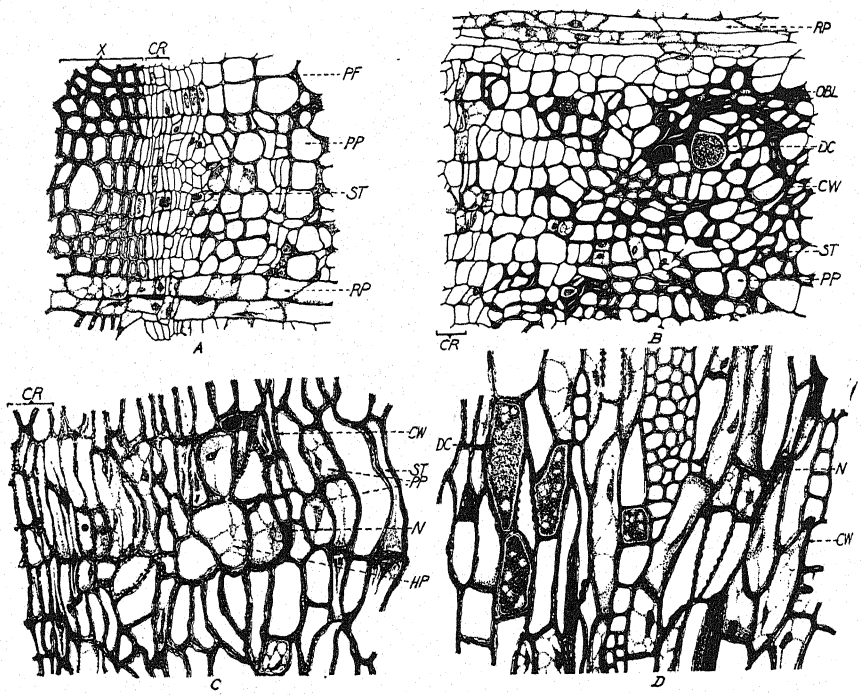


FIG. 3. Diagrammatic sketches of diseased and healthy phloem tissue from American elm stems. A. Transverse section of healthy phloem tissue. B, C, D. Transverse, radial and tangential sections, respectively, of diseased phloem tissue. Details are: CR, cambium region; DC, diseased cells; PP, phloem parenchyma; PF, phloem fibers; HP, hypertrophied phloem cell; CW, enlarged cell wall; RP, ray parenchyma; X, xylem tissue.

tissues. It is not certain whether necrosis is primary or secondary to the accumulation of the material. The discoloration is first observable in the phloem tissue of the roots. Later it may be observed in the lower trunk, sometimes being more pronounced on one side of the tree than on another. In an advanced stage of root necrosis the phloem tissue of the lower stem may have a typical dark discoloration which shades into a lighter color as it extends into the lower branches. Microscopic examinations of stained sections of twigs without discolored phloem, but taken from trees in which the discoloration was pronounced at the base revealed no distinguishing patho-

logic changes. Likewise, microscopic preparations of leaf sections taken from infected trees had no observable pathologic changes.

The discoloration seems to be due to a substance which becomes concentrated during phloem necrosis in the roots and it progresses into adjacent phloem tissue preceding degeneration and necrosis, or is formed coordinately with the destruction of tissues. It is not known whether this material is a product of the disturbed physiological processes of the diseased cells, whether it is material from the degenerated necrotic cells, or whether it might be associated with the virus entity itself. In severely discolored tissue, it closely resembles wound gum.

It is difficult to account for the wintergreen odor associated with diseased tissue, but it is apparently associated with the virus infection since the odor cannot be detected in healthy elms.

SUMMARY

Phloem necrosis is a virus disease of elm. The virus is readily transmitted by grafting diseased bark tissue to healthy suscepts. A natural vector has not been discovered.

Following infection, the fibrous roots die first. Necrosis progresses from the fibrous roots into the larger roots and finally, after death of the roots, the inner phloem in the lower portion of the stem may be killed.

Microscopic pathological changes in the primary phloem tissue of the root include hypertrophied cells and nuclei in the vicinity of mature sieve tubes, followed by hyperplasia and finally crushing of the sieve-tube cells and companion cells in the older tissues. Hyperplasia and hypertrophy of parenchyma are the most striking microscopic symptoms in the phloem of older roots and stem.

A yellow to brown discoloration of the phloem tissue accompanies the death of the roots and usually progresses into the stem and lower branches before the tree dies. Associated with the discoloration is a faint odor of wintergreen in freshly cut tissues, which is not detectable in comparable healthy tissues.

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HOST–PARASITE RELATIONS IN RED ROT OF SUGAR CANE

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(Accepted for publication April 30, 1944)

Information on how a fungus grows through the tissues of its host, how it penetrates the epidermal layers, how it again breaks out to the surface to produce its spores, and how the host reacts to the advancing mycelium is necessary to understand the disease which it causes. Cell walls, some of which are of considerable thickness, are penetrated and the protoplasm and other cell contents are changed to a greater or less extent as the fungus absorbs the nutrients essential for its own growth.

In recent years, botanists have shown considerable interest as to how cell-wall penetration takes place and there have been various theories to explain it. The usual explanations have been either that the fungus produces an enzyme which dissolves the cellulose in advance of the invasion thread or that the fungus actually exerts enough pressure to force the hypha through the cellulose wall. In the more recent literature the latter theory is perhaps the more generally accepted.

The reaction of the protoplasm to the advancing mycelium involves many complex biological and chemical problems. This concerns the questions of resistance and susceptibility and must in the future receive more consideration than it has in the past.

For many years while studying the red rot of sugar cane caused by the fungus *Colletotrichum falcatum*, now known as *Physalospora tucumanensis*, numerous sections of diseased sugar-cane tissues have been examined and it has been possible to observe the cell-wall penetration by the mycelium and to watch the changes in the protoplasm. While it is not possible to present definite evidence to support either of the theories of cell-wall penetration, or to explain the protoplasmic changes observed, much information has been obtained on what actually occurs.

Sugar cane is an extremely useful and valuable plant for studying host-parasite relationships. The plant is large and the elements in the stem and leaf are unusually well separated. The parenchyma cells in which the sucrose is stored are large and the cell walls are firm, relatively thick, and have numerous pits which are large enough to be plainly seen in ordinary freehand sections. Furthermore, sugar cane is propagated vegetatively. It has long been recognized that this type of propagation accounts to a very large extent for the severe and very devastating disease epiphytotics which have occurred on sugar cane in the past in various parts of the world. All plants of a variety, being identical from a genetic standpoint, are equally susceptible to any new parasite or any new parasitic strain of an organism when conditions are favorable for the plants to be attacked. The occurrence of such an organism and such conditions have in the past meant crop failures.

Such a plant, however, is ideal for various physiological, histological, and pathological studies.

REACTION OF SUGAR-CANE TISSUES TO RED ROT

The tissues of the sugar-cane plant react variously to the presence of the red-rot fungus. Mycelium from spores germinating in the ducts of the fibrovascular bundles or from other centers of infection grows out very rapidly through a number of layers of cells. While a certain amount of variation occurs in the growth of this mycelium, the invaded cells ordinarily do not immediately become filled with mycelium, as the mycelial threads after entering a cell usually branch but little and very commonly grow directly across the cell to the opposite wall and into the adjoining cell.

Eventually, however, there is a reaction or a change of some kind in the host cells in advance of the invading mycelium. The protoplasm changes in color and a gummy dark red material oozes out of the cells and fills the intercellular spaces (Fig. 1, C, D). It is not clear whether these modifications are merely due to the disintegrating protoplasm or not. This zone in advance of the mycelium in which the changes occur turns red, because of the presence of a soluble dye which is absorbed by the cell walls (Fig. 1, B). Under some conditions, especially in standing cane, the fibrovascular bundles may become red several inches from the center of infection. The growth of the advancing mycelium is stopped or at least checked temporarily by this red zone. Whether this is due to the presence of some counteracting toxic substance, to the plugging of the pits in the cell walls by the gummy material, or for some other reason is not clear.

When this zone develops there is then present the typical or characteristic lesion of red rot, a white or straw-colored spot with mycelium surrounded by a distinct red border with little or no mycelium. How large this lesion becomes depends largely on the natural resistance of the variety and the condition of the stalk (Fig. 1, A). The natural resistance of a variety seems to depend on how rapidly the counteracting changes occur, or, in other words, how soon the red zone around the infected area develops. In standing cane of resistant varieties, the lesions may remain very small, while in susceptible varieties they may extend entirely across the stalk. The reaction of the cane tissues, especially in resistant varieties, may be so rapid that the first invaded cells take on the red color. When this happens the lesion remains very small and often the reaction is so great that the invading mycelium is killed before it has a chance to spread materially.

The intensity of the reaction of the cane tissues is also influenced by factors which affect the functioning of the cells. In stalks which are not functioning normally, as for example stalks which have been cut and placed in a moist place, the response of the host cells to the invading mycelium is not so pronounced and often even in resistant varieties the growth of the mycelium is not greatly restricted. The interior of such stalks may be mottled but the red zones are rather indefinite and even the fibrovascular

bundles often fail to absorb any red dye. In cut stalks which have been placed in a warm situation and especially if allowed to dry to a certain extent, there is very little if any response of the host cells to the fungus. The whole interior of such stalks becomes permeated with mycelium and areas of various sizes become black in color. In these areas the fungus grows

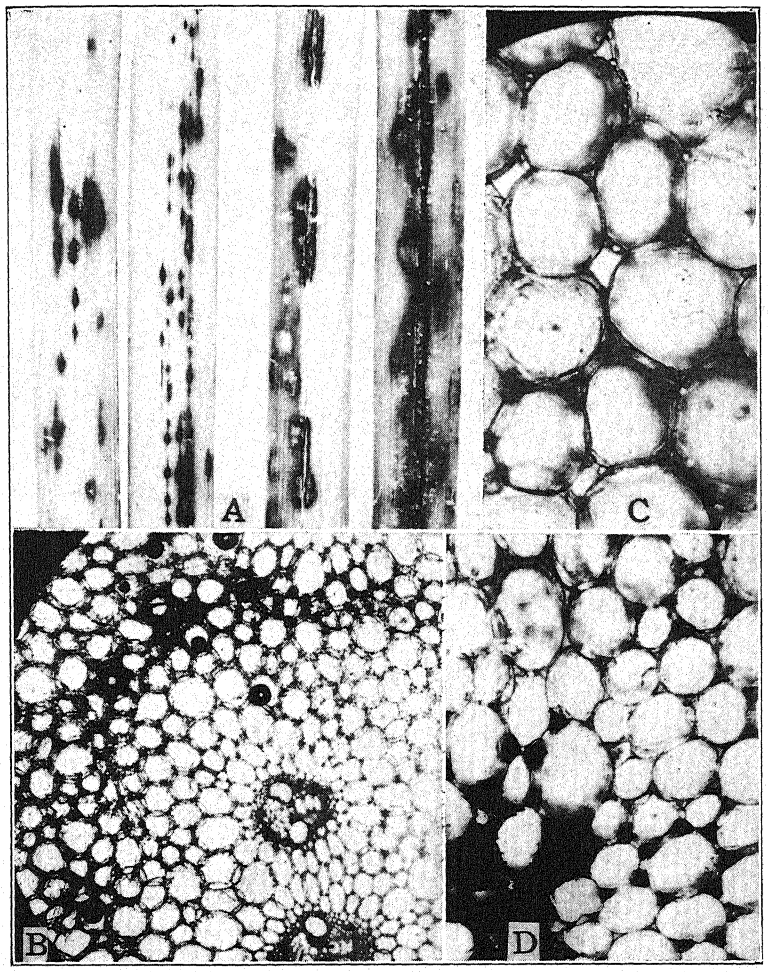


FIG. 1. Lesions of red rot in interior of cane stalk. A. Lesions as they appear in split stalks: on left, a resistant variety, Co. 281; on right, a very susceptible discarded seedling. B. Cross section of stalk through a lesion, red zone above and to the left. C. Early stage of red zone showing droplets of gummy material oozing out into the intercellular spaces. D. Later stage showing intercellular spaces filled with gummy material.

unrestricted, as it does in culture media, and the cells become filled with large hyphae which are brown in color.

The mycelium of *Colletotrichum falcatum*, then, in tissues of sugar cane varies from the relatively small and scattered hyaline threads in the newly infected regions to the network of thick, brown threads found in the old

dead areas. While the rate and extent of the growth of this mycelium depends to a certain extent on the natural resistance of the variety, this resistance can be modified and broken down by factors affecting the functioning of the host cells.

PENETRATION OF CELL WALLS IN LESIONS BY HYPHAE

Apparently in all cases in the internal parenchyma the mycelium passes from cell to cell through the pits in the cell walls. In the young, active hyphae the hyphal tip is small and swelling of the mycelium on opposite

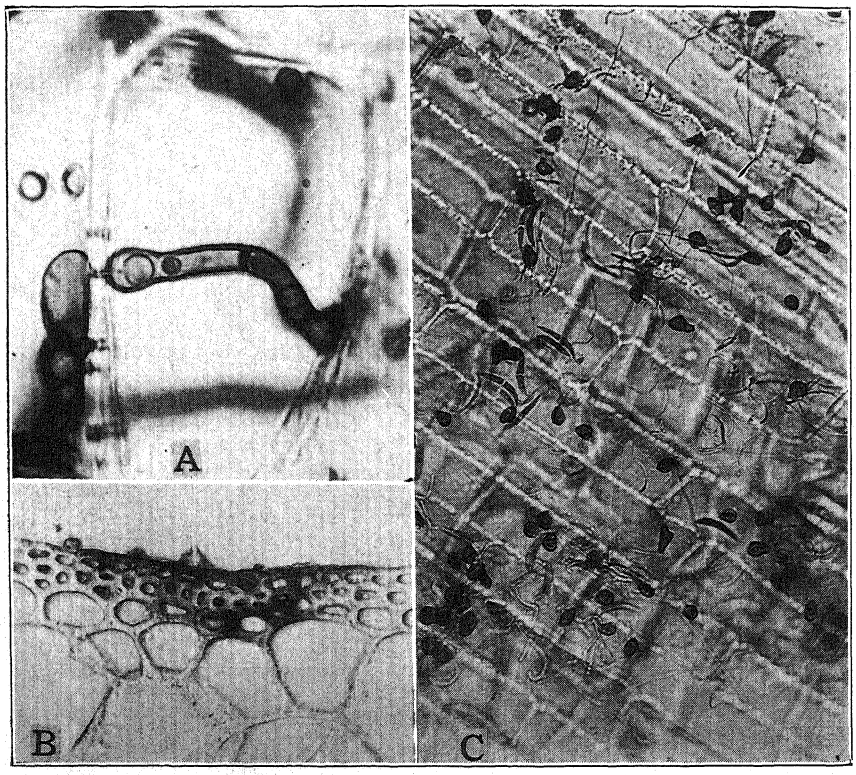


FIG. 2. A. Mycelium passing through pit in an old stalk lesion, variety C.P. 34/79. B. Cross section of red lesion on upper surface of midrib below appressoria, 17 days after inoculation, variety C.P. 34/120. C. Appressoria on inner surface of leaf sheath, variety C.P. 34/120.

sides of the cell wall where it passes through a pit is not pronounced. On the other hand, the old, brown hyphae within the cells in the black lesions are much thicker than the small segments passing through the pits. Frequently with such the hyphal thread is swollen on each side of the cross wall (Fig. 2, A). Whether this swelling takes place before the penetration of the pit has not been determined. There is, however, no evidence to indicate that there is any enlargement of the pit by any enzyme which might destroy or erode some of the cell-wall cellulose.

INFECTION THREADS FROM APPRESSORIA

Conidia of the red-rot fungus, when placed on the uninjured epidermis of the leaf or stem, germinate and produce single-celled, brown appressoria which become cemented to the substrate on which they develop. With related fungi it is known that appressoria produce germ tubes which penetrate the host tissues by going directly through the cell walls. Such a method of infection has been suggested for the red-rot fungus, but, in the past, inoculation experiments made by placing spore suspensions on the uninjured epidermis of the cane plant have in general not been convincing, and infection, except through wounds, has not been demonstrated with certainty.

To determine definitely whether the appressoria function in infection, and if so to determine how the infection occurs, a number of tests were made during the summer of 1943. Spore suspensions were sprayed on various parts of the cane plant. In spite of the fact that the weather was unusually dry while the tests were made, the conidia germinated readily and quickly produced appressoria (Fig. 2, C). The inoculated areas were examined over a period of several days for the development of lesions and numerous freehand sections were examined for the presence of infection threads from the appressoria.

In some of the inoculation tests, spore suspensions were sprayed on the upper surface of midribs. In general, satisfactory infection was not obtained in these tests. A few small lesions were observed but these did not increase in size. In sections through these lesions (Fig. 2, B) the walls of 3 or 4 layers of cells were reddened, but mycelium was not found with certainty in the cells and no infection threads were found in the thickened walls of the epidermal cells.

In other tests the spore suspensions were dropped in behind the leaf sheaths of young active leaves and allowed to come in contact with the inner epidermis of the leaf sheath and the young growing stem. The epidermal layers of these structures were tender and the cell walls were soft. On such surfaces, infection was obtained easily and rapidly. In 3 to 4 days lesions extending entirely through the leaf sheath were observed. Sometimes the whole leaf sheath later became discolored and large dead spots developed (Fig. 3, A, B). Also, numerous small lesions formed on the young stems (Fig. 3, C). While these stem lesions turned red and often penetrated 2 to 4 layers of cells, they were not observed to penetrate through the dense fibrovascular layer to the interior of the stem. In the lesions on the leaf sheaths and stems, acervuli with spores developed quickly. The infection on the leaf sheaths was similar to that which sometimes occurs in nature. Ordinarily, in the field, spores developing in acervuli on the midrib are washed down to the ligular region and, when possible, behind the leaf sheath. That more natural infection does not occur on leaf sheaths in the field is apparently due to the fact that the coating of wax makes a watertight seal between stalk and leaf sheath.

In the sections made from recently inoculated leaf sheaths, infection threads were found. These were sent out from the contact surface of the appressoria and penetrated the cell walls of the epidermis (Fig. 4). These threads were extremely small and penetrated the wall usually in a straight line and generally either perpendicular to the leaf surface or parallel to the cross walls of the subepidermal layer. In no case was any erosion observed in the cellulose wall around the infection thread, and consequently there was

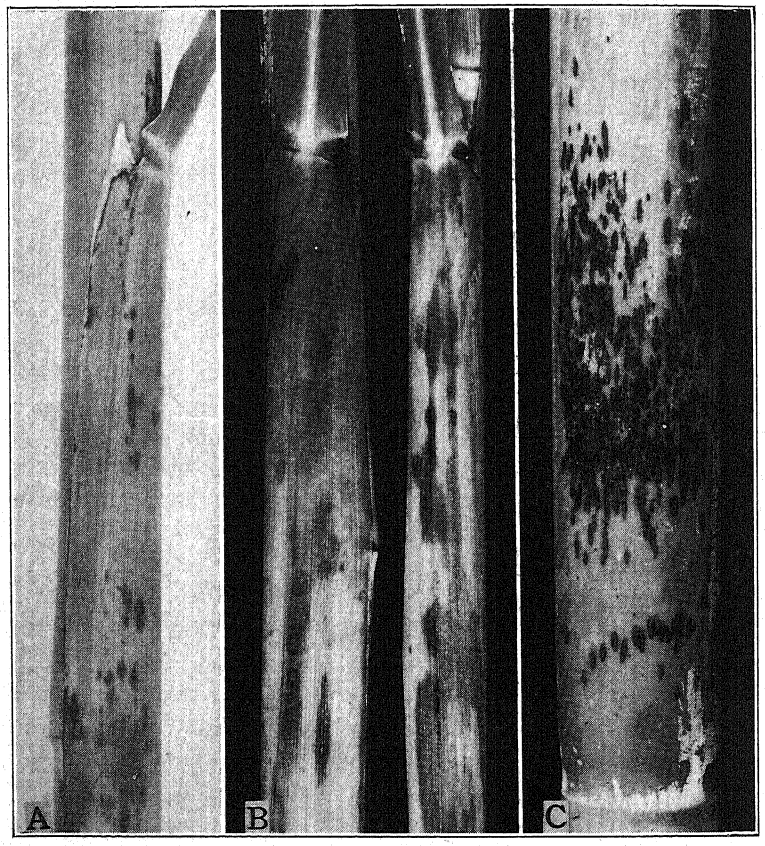


FIG. 3. Red-rot lesions from spore suspension dropped behind leaf sheath, variety C.P. 34/120. A. Leaf-sheath lesions, 10 days after inoculation. B. Leaf-sheath lesions 15 days after inoculation. C. Lesions on young stalk, 5 days after inoculation.

no evidence suggesting that the thread went through a pore dissolved out by an enzyme. These infection threads, which were easily seen when stained with cotton blue, were often quite numerous.

How the threads penetrated the wall was not determined. Pits were not observed in the outside walls of the epidermal cells. It does not seem reasonable that an extremely delicate thread could penetrate a solid cell wall by pressure without some twisting and bending. The demonstration of pores, pits, or at least lines of cleavage perpendicular to the surface, would be extremely helpful in explaining these straight infection threads.

The 2 walls of the epidermal cell on the inside of the leaf sheath are very close together, often being almost in contact. Very frequently the infection thread penetrated both walls with no apparent widening out in the very narrow lumen.

After entering a cell of the second layer, the infection threads widened out to the normal small type of hyphae. These hyphae, however, continued to grow directly towards the center of the leaf sheath, often elongating parallel to the cross walls of the cells (Fig. 4, E). Often a somewhat flattened body was visible where the infection thread first entered the interior of some of these cells. These, however, were not always present and their

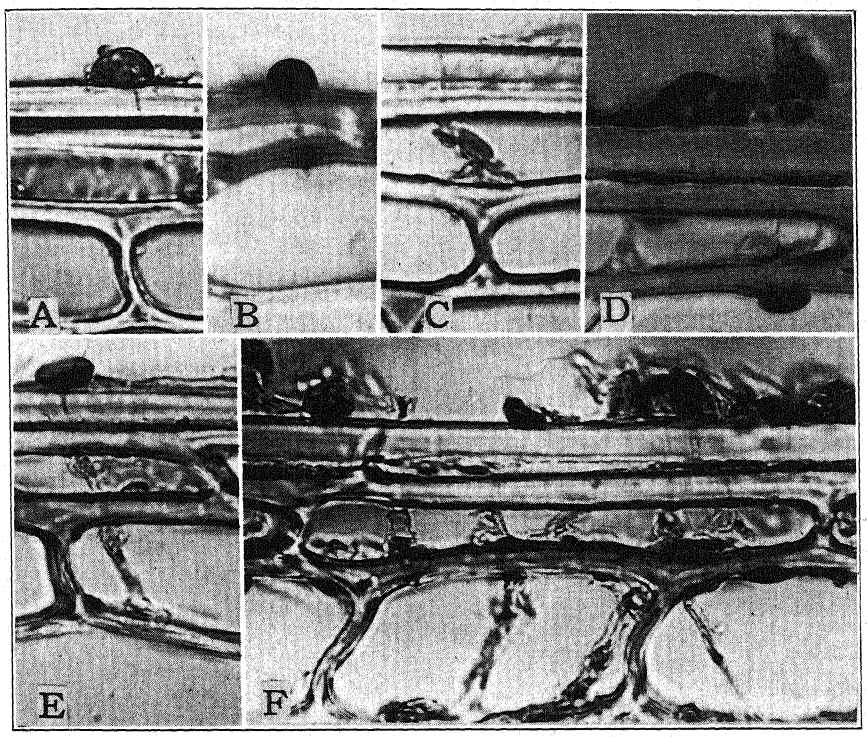


FIG. 4. Infection threads and mycelium from appressoria, variety C.P. 34/120. ×780. A. Early stage of infection thread. B. Infection thread 4 days after inoculation. C. Infection thread connected to mycelium in subepidermal cell. D. Appressoria with infection threads, mycelium, and black body. E. Infection thread and mycelium growing toward center of leaf sheath but parallel to cell wall. F. Many infection threads, several connected with mycelium.

nature was not determined. No enlargement of the hypha on opposite sides of the cell wall was observed in these newly infected cells.

In infection from the appressoria there was no indication that mycelium from an infection thread after passing through the cuticle becomes dormant between the cuticle and the cell walls of the outer layer of the epidermis. Such has been shown by Simmonds[1] for some of the anthracnoses found on

[1] Simmonds, J. H. Latent infection in tropical fruits discussed in relation to the part played by species of Gloeosporium and Colletotrichum. Proc. Royal Society of Queensland for 1940 52(2): 92–120. 1941.

mango, banana, and other tropical fruits. Whether such a condition ever occurs in the leaf blade of sugar cane has not been determined. It is known, however, that the red-rot fungus often will break out all over a leaf placed in a moist chamber, and this is characteristic of a dormant stage of a parasite.

ACERVULUS FORMATION

After growing for a period within the tissues, the red-rot fungus breaks out to the surface and produces its spores in an acervulus. Whether the

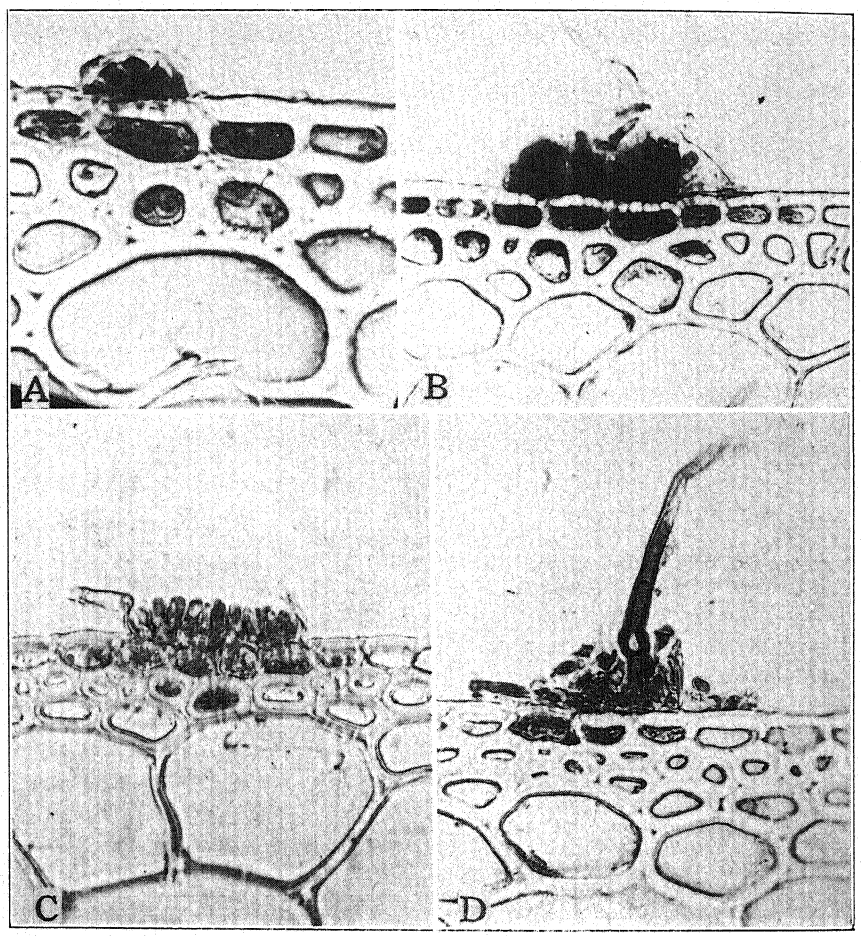


FIG. 5. Development of acervulus between cell wall and cuticle on upper surface of midrib, deep-staining mycelium in epidermal cells and fine threads through cell wall, cross section, variety C.P. 34/120. A. Young acervulus with cuticle still unbroken. ×780. B, C. Older lesions with cuticle ruptured. ×390. D. Still older stage with setae. ×390.

acervulus is a structure sufficiently developed and differentiated to be considered a fruiting body is open to argument. It is in fact nothing but a cluster of conidia-bearing conidiophores intermixed with a number of long, dark-colored setae. Both the conidiophores and setae develop directly from the mycelium in the tissues.

Acervuli are ordinarily very abundant on both surfaces of the midrib. These develop quickly and as there is very little breakdown of the midrib tissues, especially of the upper epidermis, the formation of the acervuli can be readily followed by a study of free-hand sections.

The thick, hard, membranous epidermis of the upper surface of the midrib consists ordinarily of about 3 layers of thick-walled cells which are

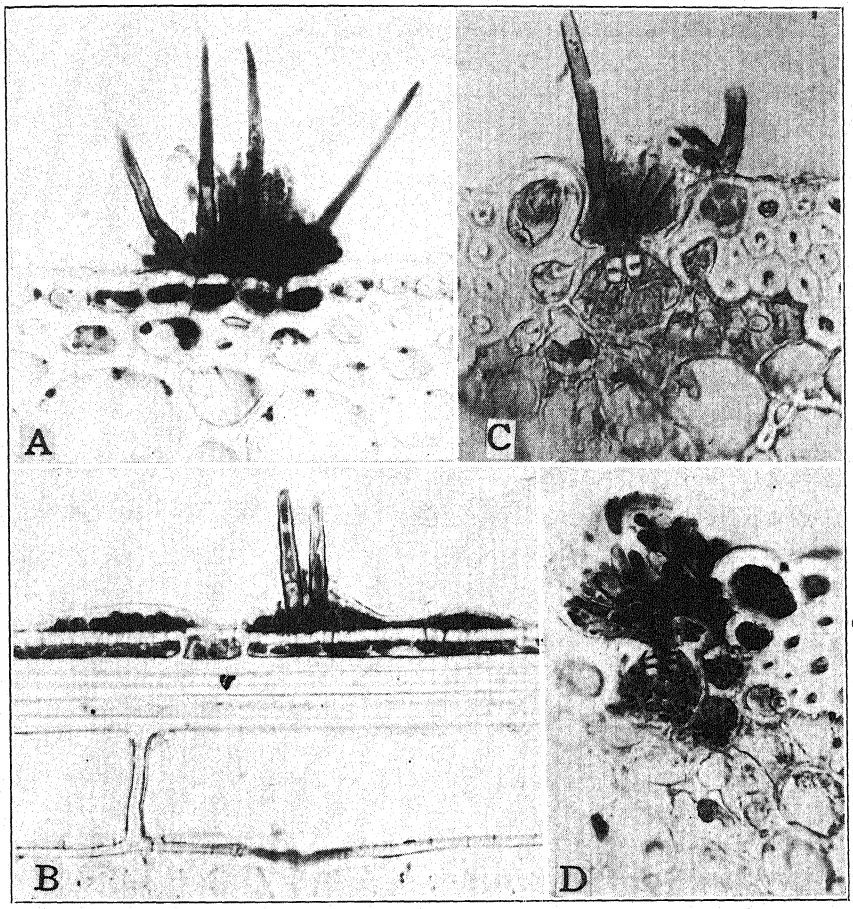


FIG. 6. Acervuli, variety C.P. 34/120. ×390. A. Old acervulus with spores, upper surface of midrib, cross section. B. Acervulus, upper surface of midrib, longitudinal section. C, D. Acervuli from stomates, under surface of midrib, showing deeply staining mycelium in epidermal cells and fine threads through cell walls.

elongated in the direction of the main axis of the leaf. In the formation of the acervuli (Figs. 5, 6), these cells and especially the ones in the outside layer, become packed with mycelium. This mycelium is very active and apparently contains much nutritive material, as it stains much more deeply with cotton blue than do the hyphae found in the interior tissues. Very small hyphae then pass through the outside cell wall from this mycelium.

This is the reverse of what occurs in infection from appressoria. Why these small threads pass inward through the cell wall from the appressoria and outward from the active hyphae in the epidermal cells is another of the unexplained host-parasite relationships. After passing the cell wall, the small threads immediately begin producing the conidiophores and setae between the wall and the cuticle (Fig. 5, A). Apparently the nutritive material necessary for spore formation comes through the fine threads from the active mycelium in the epidermal layers. As the conidiophores and setae develop, the cuticle separates from the cell wall and is pushed up until it finally ruptures (Fig. 5, B, C). This subcuticular development is characteristic of acervulus formation on the upper surface of the midrib.

On the under surface of the midrib, the mycelium, taking the easiest way, pushes out through the stomates (Fig. 6, C, D). But even here the acervulus is reinforced by mycelium which is packed in the thick-walled cells bordering the channel in which the stomates are located. Small threads pass out from these cells and produce conidiophores just as they do from the cells on the upper surface of the midrib.

Acervuli also form when only a single layer of cells is infected and even form on single-celled plant hairs which contain mycelium.

The fine threads passing through the cell walls and supplying nutrients to conidiophores for the production of conidia, in contrast to the infection threads which develop from the appressoria, function for days or even weeks. If these threads produce an enzyme which may act on the cellulose, its action should be evident. Sometimes, in sections of rather old acervuli, there has seemed to be some erosion of the cellulose around the fine threads and in a few cases it appeared as if the acervulus had broken back into the outer layer of epidermal cells but the latter cases were not common.

SUMMARY

A study was made of some of the host-parasite relationships of the red rot of sugar cane, including cell-wall penetration and the reaction of the host cells to the invading mycelium.

An internal lesion of red rot typically consists of a white to straw-colored center surrounded by a red zone.

In advance of the mycelium the protoplasm of the host cells becomes modified and a gummy material oozes out and fills the intercellular spaces. At the same time, a reddish dye-like substance is produced which is absorbed by the cell walls.

Mycelium advances but slowly into the red zone. The red zone forms quickly in resistant varieties and more slowly in susceptible ones.

In stalks in which the cells are not functioning normally, the mycelium spreads more readily than in standing cane.

In the stalk and leaf, the mycelium penetrates the cell walls through pits.

Infection threads from appressoria were observed in the epidermal cell walls of the leaf sheath.

Acervuli develop from mycelium packed in the epidermal and subepidermal layers. This mycelium sends very small threads through the walls of the epidermal cells. The conidiophores and setae develop from these threads. The acervuli are subcuticular.

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SEEDLING INFECTION OF DENT MAIZE BY SCLEROTIUM BATATICOLA TAUB.[1]

G. SEMENIUK

(Accepted for publication May 10, 1944)

INTRODUCTION

Sclerotium bataticola Taub. causes a seedling disease of many crop plants in the field and under greenhouse and laboratory conditions. Its attack on corn, as generally observed, is confined to the mature or nearly mature plants. Its parasitism on seedlings, here reported, has not been noted previously.

The charcoal-rot disease of dent maize was found in Iowa in 1941 for the first time[2] and was found again in 1943. In 1941, the disease occurred in mid-August, in only trace amounts in one field near Ames. The corn in this field was planted early and was ripening prematurely with approximately 30 per cent of the stalks dead in mid-August. The soil was Webster silty clay loam. July and August were very dry and the corn throughout the state ripened somewhat earlier than normal, particularly on the knolls where drought conditions were more severe.

In 1943, the disease was found in mid-September on approximately 33 per cent of the plants in parts of several fields in a small area, approximately 2 miles south of Cherokee, Iowa. The plants in these fields were nearly all dead or dying, approximately 50 per cent of them being broken over several nodes above the ground. The corn outside this area and throughout the state was still very green and upright. Rainfall was above normal throughout most of the State in 1943 except in the area indicated, where the rainfall was much below normal during August and September. In addition, the soil in this area was Dickinson loam having light texture with sandy to gravelly subsoil.

METHODS

Corn-seedling infection was studied in the greenhouse during the winter of 1941–42. A culture of *Sclerotium bataticola* was used which was isolated in mid-August, 1941, from the stalk of a diseased corn plant in a field near Ames, Iowa. Inoculum was prepared by growing the fungus for 3–4 weeks on 50 gm. of a 5 per cent corn-meal-soil mixture contained in 125-ml. Erlenmeyer flasks and wetted to approximately 70 per cent of its water-holding capacity. Weighed amounts of this inoculum were added to each 4-inch pot of soil for the infested series, while no additions were made to the non-infested series. The inoculum was distributed uniformly as a thin layer at

[1] Journal paper No. J-1187 of the Iowa Agricultural Experiment Station, Ames, Iowa, Botany and Plant Pathology Section, Project No. 577.

[2] Semeniuk, G. Charcoal-rot of maize, new to Iowa. (Abstr.) Proc. Iowa Acad. Sci. 49: 256. 1942.

the seed level or at some measured distance above or below it. Seven seeds were planted in each pot and usually 3 weeks were allowed for seedling development.

The soil used was 2 parts greenhouse compost to one part of washed river sand, steamed for 5 hours at 15 lb. pressure and used immediately. Non-disinfested seed of inbred line Hy was used in all the tests.

The developed seedlings were washed out and measured for: wet weights of seedlings per pot; height of individual seedlings; mesocotyl and primary root necrosis of individul seedlings. The mesocotyls were rated 0, 1, 2 or 3 for disease development indicating that they were healthy, a trace to ⅓ necrotic, ⅓ to ⅔ necrotic, or ⅔ to completely necrotic, respectively. The primary roots (radicle and seminals) were rated 0, 1, 2 or 3, indicating that there was no necrosis, one to several small necrotic areas on one or more roots, several elongated necrotic areas usually on two or more roots, much elongated necrotic areas up to complete or nearly complete necrosis of one (radicle) but usually all members of the primary root system, respectively. The disease severity for a group of seedlings was determined by the following formula:

$$\frac{\text{Sum of ratings given individual plants} \times 100}{\text{Number of plants examined} \times 3}.$$

DISCUSSION OF RESULTS

No pre-emergence rotting of seed occurred in *S. bataticola*-infested steamed soil. The seedlings developed normally for approximately 2 weeks, whereupon stunting and reduction in vigor of the seedlings became manifest and later accentuated (Fig. 1, A). Not all the seedlings in any one pot infested with *S. bataticola* were similarly affected. A few seedlings remained as vigorous as those in the non-infested pots of soil. In general, the occurrence and the extent of stunting reflected the progress of mesocotyl and primary root necrosis.

In most of the seedlings in the infested soil, necrosis was severe in mesocotyl and primary root. In the milder cases of necrosis the affected tissues were light brown and without sclerotia of *S. bataticola,* while in the more severe cases they were dark brown to near black because of an abundance of sclerotia (Fig. 1, B). The discoloration probably was due to other organisms accompanying *S. bataticola,* particularly *Fusarium moniliforme,* since many infected roots bearing an abundance of sclerotia were colorless, while discolored tissues frequently yielded *F. moniliforme* and other organisms along with *Sclerotium bataticola.* When mesocotyl necrosis was severe, the tissues were partially disintegrated and easily torn apart by the force of fine streams of water during washing. In severe necrosis of the seminal root the cortex was completely disintegrated and absent, leaving intact only the stele and epidermis.

Although sclerotia frequently were on the pericarp, mesocotyl, coleoptile, primary and occasionally secondary roots (Fig. 1, B), their absence in

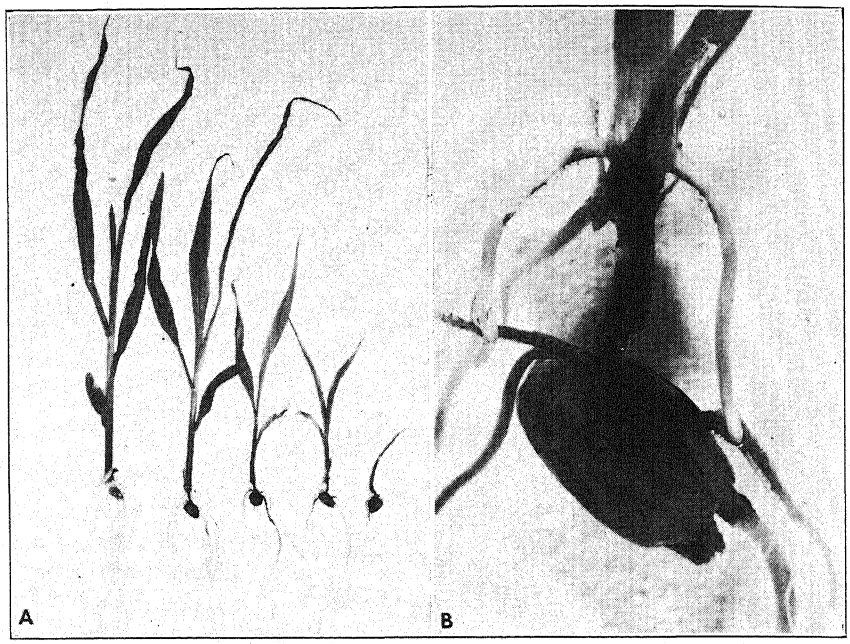


FIG. 1. *Sclerotium bataticola* on maize seedlings (inbred line Hy) after 3 weeks in infested steamed soil. A. A healthy seedling (on the left) and 4 diseased seedlings showing different degrees of stunting and disease development on the mesocotyls and primary roots. B. A close-up view of the second seedling from the right in A. Sclerotia were abundant on the pericarp of the seed, seminal roots, mesocotyl, coleoptile, and crown. The cortex and much of the epidermis of the seminal roots had disappeared.

the less affected tissues was not always indicative that the fungus was not present. The fungus always was isolated from necrotic tissues beyond the regions of sclerotia and occasionally from apparently healthy regions adjacent to necrotic areas without sclerotia. Sclerotia were observed extend-

TABLE 1.—*Disease development on maize seedlings grown in steamed soil infested with Sclerotium bataticola and held at 11.5° C. for different periods before being transferred to the greenhouse at 25° C.*

Soil treatment	Days at 11.5° C.	Seedling height[a]	Disease severity[b]	
			Mesocotyl	Roots
	No.	*cm.*		
Infested	0	32.2	58.4	60.7
	2	32.4	47.5	35.9
	4	38.8	32.1	37.1
	6	30.4	38.1	25.8
Non-infested	0	41.2	8.3	6.0
	2	34.6	7.4	6.2
	4	35.9	7.4	2.5
	6	37.0	4.8	1.2

[a] Mean of 4 replications.

[b] $\frac{\text{Sum of ratings of individual plants} \times 100}{\text{Number of plants examined} \times 3}$.

TABLE 2.—*Disease development on maize seedlings in Sclerotium bataticola-infested and noninfested steamed and nonsteamed soil with treated and nontreated seed*[a]

Seed treatment	Soil steaming	Soil infestation	Location of inoculum	Amount of inoculum	Seedling height[b]	Seedling weight	Disease severity	
							Mesocotyl	Roots
				gm.	*cm.*	*gm.*		
None	Steamed	None	.....	.....	35.7	27.8	13.6	9.9
"	"	*S. bataticola*	Seed level	5	36.4	26.6	100.0	71.5
"	"	"	"	10	37.0	25.1	96.5	64.3
"	"	"	"	20	32.6	21.3	94.1	69.1
"	"	"	Above seed level[d]	20	34.2	22.7	97.6	46.9
"	"	"	Below seed level[e]	20	30.0	23.7	96.3	66.7
"	"	*F. moniliforme*	Seed level	20	32.9	27.6	13.1	1.2
"	"	*F.m.* + *S.b.*[c]	"	20 (each)	32.4	22.6	77.4	22.6
"	Nonsteamed	None	.....	.....	35.5	24.8	15.5	4.8
"	"	*S. bataticola*	Seed level	20	33.2	20.0	9.0	1.3
Semesan Jr. (soak)	Steamed	None	.....	.....	37.8	33.3	3.6	0.0
"	"	*S. bataticola*	Seed level	20	34.4	26.8	84.6	48.8
"	"	"	Above seed level[d]	20	35.6	26.4	60.7	4.8
"	"	"	Below seed level[e]	20	35.6	26.8	83.4	45.3

[a] Greenhouse temperature approximately 25° C.
[b] Mean of 4 replications.
[c] *Fusarium moniliforme* and *Sclerotium bataticola*.
[d] 1½ cm. above.
[e] 2 cm. below.

ing along the seminal roots as much as 8 cm. from the seed. The development of necrosis began at the seed (location of the inoculum) and progressed for varying distances along one or all members of the primary root system and along the mesocotyl. The fine branch roots also became infected and eventually disjoined. Secondary roots became infected following complete necrosis of the mesocotyl during the early development of the seedling. An occasional lesion with sclerotia occurred on the secondary roots that passed down through the region where the inoculum was applied, but usually they were free of infection. The fungus also was isolated from the scutellum and from the plumular region of the more severely stunted seedlings.

The disease was more severe at 25° to 30° C., than at lower temperatures. Maintaining the seed in contact with inoculum in cold, wet (steamed) soil

TABLE 3.—*Disease development on 4 inbred lines and 2 single crosses of dent maize in steamed soil infested with Sclerotium bataticola*[a]

Maize seed	Soil infestation[b]	Seedling height[c]	Seedling weight	Disease severity	
				Mesocotyl	Roots
		cm.	*gm.*		
Inbred line 1	Infested	37.7	22.9	50.9	36.8
	Noninfested	40.4	32.1	4.8	1.6
2	Infested	32.1	23.0	63.3	26.7
	Noninfested	33.3	25.1	4.8	0.0
3	Infested	37.9	26.1	20.6	9.5
	Noninfested	39.6	36.8	3.2	1.6
4	Infested	37.8	22.8	70.0	36.7
	Noninfested	40.9	29.4	7.9	3.2
Single cross 1	Infested	38.6	31.9	79.7	48.2
	Noninfested	42.0	32.2	12.7	4.8
2	Infested	41.2	31.2	13.3	8.3
	Noninfested	44.9	38.7	6.4	3.2

[a] Greenhouse temperature approximately 25° C.
[b] 20 gm. inoculum placed at the seed level.
[c] Mean of 3 replications.

for 2–6 days before transferring to the greenhouse at 25° C. resulted in less disease under longer exposures to these conditions (Table 1).

Similar disease development occurred with applications of 5, 10 or 20 gm. of inoculum to each pot of soil or by placing inoculum 2 cm. below or 1½ cm. above the seed level (Table 2). Treating the seed by 15 minutes' soaking in a 0.001 per cent water solution of ethyl mercury phosphate only slightly reduced disease development. Less disease occurred from inocula of *Fusarium moniliforme* and *Sclerotium bataticola* mixed immediately before their application to the soil than from *S. bataticola* alone. No disease occurred when inoculum of *S. bataticola* was added to nonsteamed soil.

The activity of *S. bataticola* apparently was influenced considerably by the antibiotic activities of other soil organisms. The fungus was able, however, to grow through steamed soil, as disease occurred on the mesocotyl

when the inoculum was placed below the seed level and on the roots when placed above the seed level. The failure of seed treatment markedly to reduce disease suggests that avenues other than seed may be available for the entrance of the organism into the mesocotyl and primary roots. While diseased tissues on agar media frequently yielded *Fusarium moniliforme* and saprophytic organisms along with *Sclerotium bataticola,* such organisms were viewed only as of secondary importance.

Differences in disease development on several inbred lines and single crosses of dent maize were noted in one test (Table 3). These results suggest that there are differences in susceptibility to this disease and that the seedling reaction might be used in finding sources of resistance to it.

SUMMARY

A seedling disease of dent maize caused by *Sclerotium bataticola* Taub. is described as obtained in the greenhouse. Severe mesocotyl and primary root necrosis with accompanying stunting of seedlings was obtained only under steamed soil conditions where the suppression of *S. bataticola* by other soil organisms was reduced to a minimum. Differences amongst inbred lines and single crosses of dent maize seedlings in susceptibility to the disease were noted.

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PHYTOPATHOLOGICAL NOTES

The Use of Abrasives for Inoculating Sugar-cane Seedlings with the Mosaic Virus.—In the routine testing of sugar-cane varieties for disease resistance at the U. S. Sugar Plant Field Station at Houma, Louisiana, from 15,000 to 25,000 new seedlings are inoculated each year with the mosaic virus as the first step in the elimination of disease-susceptible ones. The Matz[1] method is used, in which the inoculum is placed in the space between the youngest expanded leaf blade and the next younger leaf that is still rolled, and a fine needle then passed 6 to 8 times through the liquid and into the basal portion of the unexpanded leaves (the spindle).

The method is usually 90 per cent or more effective with susceptible varieties, but for inoculating several thousand small plants, such as those grown from true seed, it is tedious and time-consuming. Furthermore, plants grown from true seed are too small for rapid inoculation by the needle-prick method until they are several weeks old. Fewer seedlings can be handled in a season because of not being able to move the first-inoculated, potted seedlings from the greenhouse earlier and replace them with new plants.

It was desirable to develop a method which would be equally or more effective, but faster and less tedious than the needle-prick method. The abrasion technique, devised by Rawlins and Tompkins,[2, 3] or a modification thereof, appeared suitable if a high enough percentage of infection would be obtained. A preliminary experiment was made according to the following procedure: In one series, finely ground, unwashed, white, sea-island sand of 100-mesh grade, to which inoculum was added, was picked up between the thumb and index finger and rubbed on the spindle. In another series, 100-mesh carborundum was used in the same way. Enough pressure was exerted by the fingers to rupture the tissues to the extent that they appeared to be water-soaked. No effort was made to proportion the amounts of inoculum and abrasives, but enough of the former was added to make a mixture of thin consistency. The needle-prick method was used as control. Two-month-old seedlings, 3 to 6 inches tall, of the cross Co. 281 × U.S. 1694 were used because, in the past, seedlings of this cross had shown a relatively high degree of susceptibility to the mosaic virus. There was injury to the young plants but, insofar as could be determined, this injury did not affect their normal growth to any appreciable extent. The results are presented in table 1.

Comparatively high percentages of infection were obtained with both sand and carborundum as abrasives, the percentage of plants in each in-

[1] Matz, Julius. Artificial transmission of sugar-cane mosaic. Jour. Agr. Res. [U.S.] 46: 821–839. 1933.

[2] Rawlins, T. E., and C. M. Tompkins. The use of carborundum as an abrasive in plant virus inoculations. Phytopath. 24: 1147. 1934.

[3] ———— and ————. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. 26: 578–587. 1936.

stance being more than 3 times as great as by the needle-prick method. In other tests, comparable infection was also obtained by rubbing only the last expanded leaf with sand and inoculum. It was recognized, however, that in using as test plants those from a seedling progeny that differed genetically in their susceptibility to mosaic, strictly comparative data for the methods could not be obtained.

The results were highly encouraging and warrant further testing of the abrasion method of inoculating young seedlings. If in further comparative trials the percentages of infection obtained approximate those obtainable with the needle-prick method when it is used with older plants, then the abrasion method may very advantageously replace the method now used. One man could inoculate by the abrasion method approximately as many plants in a day as four men could by the needle-prick method.

In other experiments with plants about two months old, which were grown from cuttings of a susceptible variety, the results were not consistent.

TABLE 1.—*Results of inoculating sugar-cane seedlings with mosaic virus by the abrasion and needle-prick methods. Readings made 4 weeks after inoculation*

Method of inoculation	No. plants inoculated	Plants infected	
		No.	Per cent
Sand	50	22	44
Carborundum	50	21	42
Needle-prick	100	13	13

Infection was obtained by the abrasion method in some tests but not in others. It appeared that maturity of tissue and possibly particle size and cutting qualities of the abrasives influenced the results, and that these factors must be considered in further developments of the method, particularly with plants grown from cuttings.—DOUGLAS C. BAIN, United States Sugar Plant Field Station, Houma, Louisiana.

A Method of Producing an Epiphytotic of Tomato Fruit Rot in the Field.[1]—Fruit rot of tomato (*Lycopersicum esculentum* Mill.), caused by *Phytophthora capsici* Leonian, has been responsible for severe losses in Colorado canning areas during seasons when weather conditions were favorable for the development of the disease.[2] To determine the effectiveness of certain fungicides in controlling the disease and to test certain tomato varieties and types for resistance, field plots were planted at Fort Collins in 1942 and 1943. Because of the probability that the field soil there might not contain a concentration of *Phytophthora capsici* sufficient to bring about an epiphytotic of tomato fruit rot, a method was devised to produce sufficient sporangia for large-scale field inoculations.

[1] Published with the approval of the Director as Paper No. 182, Scientific Journal Series, Colorado Agricultural Experiment Station. The writers wish to express their appreciation to E. W. Bodine, Associate Plant Pathologist, Colorado Agricultural Experiment Station, for his assistance in the preliminary phases of this study.

[2] Kreutzer, W. A., E. W. Bodine, and L. W. Durrell. Cucurbit diseases and rot of tomato fruit caused by *Phytophthora capsici*. Phytopath. 30: 972–976. 1940.

In preliminary laboratory trials isolates of the fungus obtained from naturally infected tomato fruits were grown on steamed corn meal, steamed barley, steamed potato cubes, steamed pepper fruits, oatmeal agar, potato-dextrose agar, in water culture with pieces of fresh green and red tomato fruits, pepper fruits and stems, and in 1 per cent peptone solution. Few or no sporangia were produced by the fungus during 40 days at 25° C. on the solid media. Although sporangia were produced in most of the water cultures they were not in sufficient quantity to supply the needed inoculum.

The method finally developed was as follows: After the fungus was grown on steamed barley in 250-ml. Erlenmeyer flasks for 20 to 60 days at 25° C., the contents of each flask were passed through a food grinder and thoroughly mixed into the upper 3 inches of unsteamed field soil in a wooden flat. The soil then was watered thoroughly and covered loosely with paper to prevent excessive moisture loss. Since earlier tests had shown that soil aeration was necessary for satisfactory sporangial production, the soil was turned with a trowel once or twice daily. At the end of 24 and 48 hours sporangia were abundant. In order to determine whether there were sufficient sporangia to produce adequate inoculum in the form of swarmspores, samples of approximately one cubic inch of the soil mixture were placed in 400-ml. beakers containing about 30 ml. of tap water. Care was taken not to cover the soil completely with the water. After 1 to 3 hours at 25° C., abundant swarmspores were in each drop of the water examined microscopically. Swarmspores so produced readily caused infection. When drops taken from one of the beakers were placed on the uninjured surfaces of either green or red tomato fruits which were then incubated in a moist chamber at 25° C., infection was observed within 24 to 48 hours. Isolations from infected areas invariably yielded cultures of *Phytophthora capsici.*

To obtain adequate material for field-soil inoculations in 1942, isolates of *Phytophthora capsici* were grown for 60 days on steamed barley in one-quart milk bottles. One hundred and ten of these cultures then were ground and mixed with approximately 1⅓ cubic yards of unsteamed soil in a large wooden frame adjacent to the test field. The inoculated soil was moistened with a fine spray and covered with paper. At 8- to 12-hour intervals the soil was shoveled over to insure adequate aeration. Water was added when necessary to maintain the moisture content of the soil-inoculum mixture. At daily intervals from the third to the eighth day following its initial preparation a shovelful of the soil-inoculum mixture was placed in each of several 4-gallon glazed earthenware jars containing approximately a gallon of ditch water. Drops of water taken from these jars were examined microscopically every 30 minutes for 3 hours. Abundant swarmspores were present in these random drops in from 1½ to 2½ hours after exposure at 75° to 85° F.

In inoculating the soil where the test plants were growing, the prepared soil-inoculum mixture was spread thinly at the bases of the plants during

irrigation and shovelfuls of the inoculum were also placed at the edges of the running water at 25-yard intervals in the irrigation furrows. The soil was irrigated by the furrow method at sufficient intervals for the next 10 days to keep the surface moist. In addition an overhead sprinkler was used for the first three days following the soil inoculation. More than an acre of soil in which approximately 2,500 tomato plants were growing was inoculated in 1942. In 1943, this same method was used to inoculate the soil in field plots in which approximately 600 plants were growing. An overhead sprinkling system was substituted for the furrow irrigation used the previous season. This large-scale method of inoculation resulted in uniform infection of tomato fruits over the entire field plots in 1942 and 1943.—W. A. KREUTZER AND L. R. BRYANT, Colorado Agricultural Experiment Station, Fort Collins, Colorado.

Tomato Seed Treatment with New Improved Ceresan Dust.[1]—Ethyl mercury phosphate has been used successfully by New Jersey seedsmen for treating seventy to eighty thousand pounds of tomato seed annually during the past six years. The seed is immersed for 5 minutes in a 1–1200 suspension of New Improved Ceresan (5 per cent ethyl mercury phosphate), allowed to drain for 25 minutes, then centrifuged and dried. Samples of treated seed plated on potato-dextrose agar are sufficiently free from surface-borne organisms to meet the rigid requirements of the Georgia Department of Entomology for producing Georgia certified plants. In addition the seed remains free from contaminating organisms for an extended period of time and is protected against seed decay and damping-off organisms, benefits which are not obtained from the mercuric chloride dip previously used.

Though both the material and the method now in use are excellent for disinfesting the seed, an equally effective treatment which is less laborious and less toxic to those who treat and handle the seed would be desirable. In an effort to find a substitute material, 37 fungicides, including some of the newer materials such as Arasan, Spergon and Fermate, were tested but none was found which gave seed protection and at the same time would meet the Georgia plant certification requirements. However, a simplified method using New Improved Ceresan as a dust shows definite promise. This method, in which a dosage of 0.5 per cent by weight is used and the seed agitated for 5 minutes, has consistently given 98 to 100 per cent clean seed in many laboratory plating tests.

No seed or seedling injury has been observed in any greenhouse experiments in which a 0.5 per cent dosage has been used. Furthermore, treated seed (0.5 per cent dosage) held for 12 weeks in stoppered test tubes which prevented the escape of the ethyl mercury phosphate fumes, showed no injury when planted in soil in the greenhouse. Slight seedling injury has been noted in pure sand at dosages of 1 per cent and higher, and in soil at 2 per cent and higher.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Plant Pathology.

Plating tests show that the dust treatment prevents recontamination of seed held in cheesecloth bags exposed to air contaminants for as long as 28 weeks; and in all germination tests the dust method has been equally as effective as the dip method in preventing seed decay and damping-off.

Forty pounds of seed representing four lots have been treated in our laboratory and composite samples have met the standards required for production of Georgia certified plants. Of the four samples submitted to the Georgia Department of Entomology, two were 100 per cent clean, and the other two, 99 per cent. These lots of seed are being planted in Georgia in the spring of 1944 to test the relative effectiveness of the dust and dip methods.

The effectiveness of a commercial machine duster in applying New Improved Ceresan dust to 100 pounds of tomato seed has been tested. Nine 100-seed samples taken from three machine-dusted lots were plated and found to have only seven contaminated seed. Of these, five samples were 100 per cent clean, and no more than 2 per cent fungus- or 1 per cent bacterial-contaminated seed were found in any of the other four samples.

In all experiments so far the dust method of applying New Improved Ceresan to tomato seed has proved to be as effective as the dip method in surface-sterilizing the seed, preventing recontamination, and protecting the seed and seedling against decay and damping-off organisms. A dosage of 0.5 per cent by weight of seed, applied either by laboratory methods or by means of a commercial machine duster, has consistently produced 98 to 100 per cent clean seed with no apparent seed injury in any experiments. This promising method decreases the danger of chemical injury to workmen and eliminates the time-consuming and laborious dipping, centrifuging, and drying processes necessitated by the liquid method.—B. H. DAVIS and C. M. HAENSELER, N. J. Agricultural Experiment Station, New Brunswick, New Jersey.

THE UNIMPORTANCE OF COTTON SEED IN THE DISSEMINATION OF VERTICILLIUM WILT IN CALIFORNIA[1]

B. A. RUDOLPH[2] AND G. J. HARRISON[3]

(Accepted for publication March 30, 1944)

Verticillium wilt of cotton was first reported in California by Shapovalov and Rudolph in 1930 (11) following the isolation of the causal fungus (*Verticillium albo-atrum* R. and B.) from diseased plants grown at the U. S. Cotton Field Station at Shafter. Herbert and Hubbard (6) claim to have observed the disease in 1927, which is probably true, since the symptoms are very characteristic and not likely to be confused with those of other cotton diseases in California. Today Verticillium wilt is recognized as a destructive disease in virtually all cotton-producing sections of the San Joaquin Valley. In some fields the incidence of infection is virtually 100 per cent, and much of the cotton harvested from such fields is of poor quality.[4]

Because of the seemingly rapid spread of Verticillium wilt throughout all cotton districts of the State in comparatively few years, it was only natural that growers and plant pathologists alike should suspect seed transmission of the parasite. Investigators in Texas and Arizona, where this disease has spread with equally spectacular rapidity, have already reported seed transmission of wilt. Unfortunately, there is no agreement as to how, when, or where the seed becomes infected or contaminated by the fungus. Thus, Taubenhaus, in Texas, reported isolating *Verticillium* from the interior of 8.3 per cent of 1440 seeds in 1936 (12) and from 2.3 per cent of 1600 seeds in 1937 (13). In a personal letter to the senior writer, Taubenhaus particularly stressed the point that the fungus inhabited the interior of seeds and not the lint. He not only delinted the seed with sulphuric acid but also disinfected it with a mercuric solution. He said that no recommendations were being made (presumably to growers) until the work could be replicated 2 or 3 years. He pointed out that "one year the infection in the interior of the delinted seed is present and another year it may not be."

In contradistinction to the results obtained by Taubenhaus are those of Brown[5] (1) in Arizona. He was unable to isolate the fungus from the interior of the seed but claims to have succeeded in isolating it from the lint. He has given no details of his experiments, and no one else has made such claim. Brown (2) has reported instances of the disease appearing in

[1] Much of the work reported in this paper was done under W.P.A. Projects 50–1859, 50–11992, and 50–12456–D.

[2] Associate Plant Pathologist in the California Agricultural Experiment Station.

[3] Agronomist, United States Department of Agriculture.

[4] In addition to ripening prematurely, the bolls on affected plants are stunted, and the lint is of inferior quality as to fiber length and strength. Studies at the U. S. Cotton Field Station at Shafter confirm these observations.

[5] Although this report is not signed, it and similar articles that appeared in Arizona newspapers, such as the Arizona Star (Tucson), released to the press by the University of Arizona, credit Dr. Brown and his associates with the work.

clean fields planted to seed that had not been delinted, but he knew of no instance where the disease had developed in clean fields planted to acid-delinted seed. Presley,[6] on the other hand, has reported the appearance of the disease in desert areas of Arizona, cropped for the first time and planted with acid-delinted seed.

In letters to Miles (8, 9), Sarejanni has expressed the belief that Verticillium wilt is seed-borne, but he was unable to demonstrate the presence of the fungus in seed produced on diseased plants. Sarejanni first observed the disease near Copias, Greece, in September, 1932, in cotton plants grown from seed imported from North Carolina that same year. Seed obtained in 1932 from Virginia produced healthy plants, but it is not certain that the seed purchased in Virginia actually was grown there. The disease has never been reported or recognized in either of those States, according to letters received in November, 1942, from pathologists of the North Carolina and Virginia Agricultural Experiment Stations.

Miles (8) likewise was unable to demonstrate the presence of the parasite within cotton seed.

SEED STUDIES IN CALIFORNIA

Experiments to determine whether *Verticillium albo-atrum* is transmitted by cotton seed and whether infected seed is an important factor in California, were made from 1934 through 1938.

Each year mature cotton plants in the most advanced stages of Verticillium wilt were dug at intervals at the United States Cotton Field Station at Shafter, California, where the writers are collaborating in the production of a Verticillium wilt-resistant cotton suitable to California. Cultures of the diseased plants were made at the University of California Deciduous Fruit Field Station at San Jose, California. The plants grown at Shafter had been artificially infected by injecting pure cultures of the fungus into the soil about the roots.

Internal Seed Studies. In 1934, to determine whether the fungus is capable of infecting the internal structure of the seed by way of the vascular system, sections of the stele of each plant near the roots were cultured on Czapeck's nutrient agar, slightly acidified with lactic acid to suppress bacterial growth. At the same time, varying numbers of mature, unopened bolls, particularly those on the lowest branches, close to the main stalk, were clipped off and each put in a paper bag and kept in a dry place at room temperature.

Pending the outcome of the cultures made from the steles, Petri-plate cultures were made from either the receptacle or placentae of each boll to determine whether they were diseased. Bracts were completely removed, then the receptacle was sliced off close to the base of the boll. Next, the

[6] Presley, John T. The occurrence of Verticillium albo-atrum wilt on cotton grown as a first crop in certain remote desert areas. Unpublished manuscript.

This paper was read before the Pathological Section of the Association of Southern Agricultural Workers at Memphis, Tenn., 1942. References to and quotations from it have been made with the author's consent.

receptacle and its short peduncle were scrubbed with a brush and lightly scraped with a scalpel to remove any dirt or dead cuticle adhering to them. After a final rinsing with tap water the preparation was surface-sterilized in mercuric bichloride solution 1/1000 for 3 minutes, rinsed with sterile, distilled water, and cut into at least 3 pieces, the usual precautions to insure asepsis being taken at all times. Plantings were made in Petri plates of slightly acidified Czapeck's nutrient agar. A plate never was discarded in less than 3 weeks from the time the cultures were made, unless the fungus could be identified earlier, microscopically.

Cultures were made from the placentae of many bolls as follows: The bracts and receptacle of each boll were cut away and discarded. The carpels of the mature, unopened bolls were cut apart with a knife drawn along the sutures and worked free from the unexpanded locks by hand. The locks, after properly identified, were placed in paper bags and kept in a dry place at room temperature, pending the results of the cultures. The axile and numerous placentae of cotton form a central column that can easily be broken into its component parts with the fingers. Before doing this, however, the numerous tough, parchment-like walls that are attached to the placental column and divide the boll into locules were cut away. The individual placentae, from which the locular wall tissue had been trimmed away, were surface-sterilized in mercuric-bichloride solution 1/1000 for 3 minutes, after which they were rinsed in sterile distilled water and planted in Petri plates of slightly acidified Czapeck's nutrient agar. Figure 1 shows the successive steps taken in preparing both the receptacle and placentae for culture purposes.

Finally, cultures were made from the seed of 28 of the bolls, the receptacles of which had yielded *Verticillium* in culture. The seed was delinted either with sulphuric acid or by scraping it with a scalpel until all traces of fiber were removed. After rinsing in tap water, it was surface-sterilized by immersion in mercuric-bichloride solution 1/1000 for 3 minutes, then was rinsed with sterile distilled water. Each seed was then cut transversally into its micropilar and funicular halves which were planted in Petri plates of slightly acidified Czapeck's nutrient agar. To maintain the identity of each seed, the halves were planted in proper sequence on either side of a line drawn across the bottom of the plate with a wax pencil, the funicular halves on one side and the micropilar on the other. Not more than 4 seeds (8 halves) were planted to a plate. None of the 763 seeds cultured yielded *Verticillium*.

The results obtained in 1934 are summed up in table 1.

To determine what other parts of the boll might become infected, cultures were made from the carpels of 5 bolls, of which either the receptacles or placentae had yielded *Verticillium* in culture. The tissue was cut into fragments approximately one-half inch square, washed, and surface-sterilized as described. The tissue was found to be sterile.

Cultures also were made from the bracts of 14 bolls selected from 5 plants that had been proved to be diseased by cultural methods. The fungus had

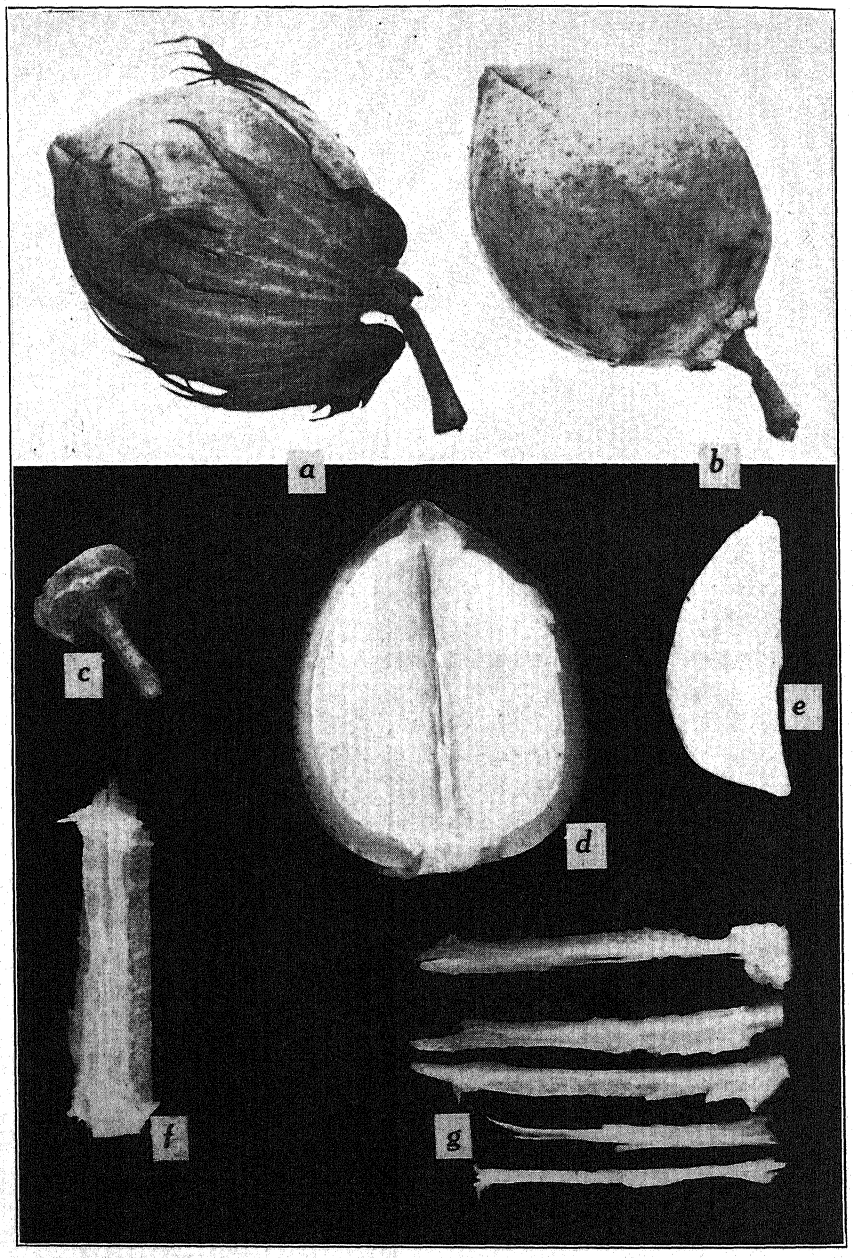


FIG. 1. Acala cotton boll and its component parts. Natural size. a. A mature boll with leafy bracts. b. A mature boll with bracts removed. c. The receptacle, trimmed and ready for sectioning and culturing. d. Longitudinal section through a boll showing the central placental column and core at its base. e. Individual lock of mature, moist, unexpanded cotton. f. Central placental column with parchment-like membranes separating the locks cut away. g. The placentae, which make up the central placental column, separated. Note core attached to the base of the top placenta. The second and third placentae are in position to show the tooth-like protuberances to which the seeds were attached.

TABLE 1.—*Results of cultures made in 1934 from either the receptacles or placentae, as well as the seed of Acala cotton plants severely affected with Verticillium wilt*

No. diseased plants	23
No. bolls from diseased plants cultured	139
No. bolls yielding no *Verticillium* from either receptacles or placentae	98
No. bolls with healthy placentae	71
No. bolls with healthy receptacles	27
No. bolls yielding *Verticillium* in culture	41
No. bolls with diseased placentae	1
No. bolls with diseased receptacles	40
No. seed cultured[a]	763
No. healthy seed	763
No. diseased seed	0

[a] From 28 bolls only, including the one with diseased placentae. Time did not permit culturing seed of the remaining 13 bolls with diseased receptacles.

reached the receptacles of 13 of these bolls. Of the latter, 3 had diseased bracts. Although the receptacles of the 14th boll failed to yield the fungus in culture, the bracts gave positive evidence of its presence. Recently dried or green bract tissue yielded the fungus in equal proportions.

In 1935 and 1936 cultures invariably were made from both the receptacle and placentae of each boll, to determine the ratio between placental infections and receptacle infections. The seed was delinted with sulphuric acid. Only seed from bolls with diseased receptacles or placentae were cultured (Table 2).

Since the accumulated data showed that the fungus only occasionally reaches the receptacles, in 1937 and 1938 cultures were made first from the receptacles. If a receptacle culture was positive, cultures were then made from the placentae of the same boll. This made it possible to work with a much greater number of bolls (Table 2).

External Seed Studies. Several hundred cultures were made from Acala cotton seed deliberately selected from the bottom of pickers' bags where the lint and fuzz were most likely to become contaminated with soil-

TABLE 2.—*Results of cultures made 1935–1938, inclusive, from both the receptacles and placentae, as well as the seed, of Acala cotton plants severely affected with Verticillium wilt*

Year	No. of diseased plants	No. of bolls cultured	No. bolls with diseased receptacles	No. bolls with diseased placentae	No. of seed cultured	No. of diseased seed
1935	12	61	1	0	?[a]	0
1936	53	434	7	1	144[b]	0
1937	101	671	2	0	34[c]	0
1938	137	2066	100	0	2961	0
Totals	303	3232	110	1	3139	0

[a] The seed count of this single boll was overlooked when the plates were discarded. Cultures made from the seed were sterile.

[b] From only six of the seven bolls with diseased receptacles. The seed from the single boll with diseased placentae was accidentally discarded before cultures of it were made.

[c] From only one of the two bolls with diseased receptacles. The other boll was badly decayed by *Alternaria* sp. while waiting the outcome of the receptacle cultures.

inhabiting organisms, without treatment of any kind. The plants that produced the seed were severely affected with the wilt, having been grown in soil artificially infested with the fungus.

The fiber on the seed was dusty, but the great bulk of plant débris had been removed in the ginning. Usually, only one seed was planted to a plate of slightly acidified Czapeck's nutrient agar. *Verticillium* failed to develop, but other fungi, too numerous to mention, quickly overran the plates.

MICROSCLEROTIA

Verticillium albo-atrum occasionally produces microsclerotia on old raspberry canes (4, 5, 10), apricot (3), and prune twigs (10). How long those that find their way to the soil remain viable is not known. At the Deciduous Fruit Field Station they have withstood desiccation in culture tubes for 10 years. Produced in any considerable quantity on old, dead cotton stalks left in the field to overwinter, it is conceivable that eventually such a large population of them might build up in the soil that contamination of the lint in pickers' bags dragged across the ground would be practically inevitable. Seed with lint thus contaminated might disseminate the fungus.

To determine whether *Verticillium albo-atrum* produces microsclerotia on dead cotton plants, experiments were started in 1937 and concluded in 1942. Each year, a patch of artificially infected plants that had developed the disease in severe form was left untouched throughout the winter at the United States Cotton Field Station at Shafter. Plants were dug and forwarded to the Deciduous Fruit Field Station at San Jose only when it became imperative to put the ground in order for spring planting.

The plants, including the main stalks and branches, were cut into convenient lengths of about 6 inches, and examined under the microscope for any kind of minute, black body that might possibly be a microsclerotium of the parasite. Representative samples of all such bodies, regardless of their origin, whether erumpent or wholly superficial, were picked up with needles and transferred to Petri plates of Czapeck's nutrient agar, some of which were acidified and some not.

Prior to making the cultures the twigs were either left untreated or were surface-sterilized by immersion in mercuric bichloride solution 1/1000 for periods ranging from 1½ to 3 minutes, and then rinsed with sterile, distilled water. The number of plantings per plate varied from 5 to 8. A total of 11,723 cultures was made during the 5-year period. Neither *Verticillium albo-atrum* nor any of its closely related forms developed in any of the cultures made.

LONGEVITY OF THE FUNGUS IN DEAD COTTON STALKS

Each year many cultures were made from the wood of the old plants used in the microsclerotia studies, to determine to what extent the parasite lives over in the main stem and twigs. Only infrequently could the fungus

be cultured, which indicates that it dies fairly rapidly in the tops of the plants, probably after they have been killed by frost. The fungus could be cultured fairly easily, however, from the heavier woody portions of the main stem at ground level and from the tap root in which decay was not too far advanced.

COTTON FIBER INOCULATIONS

To determine whether *Verticillium* can attack mature cotton fiber, either dry or wet, inoculations were made as follows: Locks of dry cotton were removed from the bolls and seeded by hand. The fiber was then fairly loosely packed in standard culture tubes, to some of which distilled water had been added in varying amounts. The tubes were plugged, steam sterilized in an autoclave, and inoculated with large fragments of sclerotial crusts of *Verticillium albo-atrum* lifted from the surfaces of cultures grown on Czapeck's nutrient agar. Such fragments simulated the occasional bits of dried bract or carpel tissue that occasionally cling to the seed after ginning, and probably even surpassed them in infectiousness.

Only cotton that was wet almost to the point of complete saturation was attacked. It turned a dark-grey, due to the presence of myriads of individual, black microsclerotia clinging to the fibers. The latter were not perceptibly discolored. The fungus failed to grow on the cotton in tubes to which no water had been added, and did not grow above the water line in those containing only small amounts of water. Presumably the humidity in such tubes was relatively high, but insufficient to enable the fungus to grow.

DISCUSSION

The considerable data obtained contribute nothing to support the theory that cotton seed from plants affected with Verticillium wilt may harbor the parasite internally. During the 5-year period of the experiments, a total of 3371 bolls from 326 plants, experimentally proved to be severely affected by the disease, were cultured. The fungus had reached the receptacles of only 150 of these bolls, and of the latter, only 2 showed infection of the core at the base of the placental column (Tables 1 and 2; Fig. 1, d and g). In figure 1, g, the core-like tissue referred to is shown attached to the first placental strip. The fungus grew only from this core-like tissue and never from the true placentae where the seeds were attached.

The original point of attachment between the funiculus and placenta is characterized by a tooth-like structure on the latter. The second and third placentae from the top in figure 1, g, clearly show these tooth-like points of seed attachment. Considering the fact that the vascular system is highly developed at these points it is not unreasonable to believe that had the fungus reached them it would have grown out and established colonies in the agar. Such was not the case.

The seed from the single boll, penetrated by the parasite in 1936 (Table 2), we regret to state, was accidentally discarded before cultures were made

of it. It is virtually certain that the seed was not infected, since the fungus never penetrated beyond the core at the base of the placentae. The seed from the boll, penetrated by the fungus in 1934 (Table 1) was cut in halves and cultured. It was not infected.

The average number of fully developed, viable seed per boll in healthy Acala cotton in California is 40. That of bolls from plants severely attacked by Verticillium wilt averaged only 32, the bolls being puny and stunted as a result of the disease. The 3371 bolls cultured, therefore, had a possible content of 107,872 seeds, all of them apparently free from Verticillium infection, since the experiments were so conducted that a check of the progress of the fungus in the stem, receptacle, and boll itself was possible. Actually, a total of 3902 seeds from bolls with infected receptacles were cut in half and cultured. All were healthy.

The late Dr. Taubenhaus (11, 12) probably is the only investigator to claim isolation of *Verticillium* from the inner tissue of cotton seed. Neither of his brief reports gives the details of his experiments. Yet he, himself, in personal correspondence with the senior writer, expressed doubt as to his findings. Pending further studies he preferred to make no recommendations to growers in Texas.

The writers do not wish to infer that infection of the inner tissue of cotton seed is impossible; but in view of accumulated negative data, it is improbable. It is conceivable that, under conditions ideal for the fungus, it may succeed in penetrating the inner tissue of an occasional seed, but this fact would have no practical significance. In the face of existing evidence other means of dissemination assume far greater importance.

Finally there is no assurance that the parasite having reached an occasional seed would remain alive there and attack the seedling the following spring. Such parasitized seed might not even germinate. It is conceivable that the fungus might be introduced into the soil with such seed. But, in the strict sense, it is wrong to assume that the disease is seed-borne without first planting such infected seed in healthful soil and observing whether it produces diseased plants.

The experiments to isolate the fungus from the fiber and fuzz left on the seed after ginning were discontinued primarily because of the nature of the disease itself and the basic habits of the parasite. Regardless of severity of wilt in the plant, the cotton in the boll is never attacked. It is true, the bolls ripen prematurely in diseased plants and are stunted and puny, but the lint is never decayed or even discolored. Since the lint is produced on the outside of the seeds, and the latter do not harbor the parasite, then contamination of the lint from outside sources could be the only source of danger. Logically spores or microsclerotia might be suspected of contaminating or even attacking the lint. Brown thinks this is possible, according to a letter to the senior writer.

Microsclerotia were not produced on the old, dead plants within a year; and even if plants were left in the fields a second year, it is doubtful whether

production of microsclerotia on the stalks would reach significant amounts in view of the comparative rapidity with which the fungus dies in the old wood. Therefore, the danger of the lint becoming contaminated with microsclerotia as the pickers drag their bags over the ground would seem negligible.

Verticillium albo-atrum, which is now known to attack over 200 widely unrelated hosts, sporulates readily in the saturated atmosphere of a culture tube, but rarely, if ever, in fields in California. There are virtually no references to spore production on field-grown plants in literature on the disease, and only occasional references to spore production on dying plants in hot frames. Van der Lek (7) describes the appearance of spores as "a very fine dew" on the necrosed leaf areas of cucumber plants growing in hot frames. Rudolph (10) also cites the comparatively few workers who have referred to spore production within the ducts and mesophyll of affected plants. Spore production on cotton plants in the field, in California at least, can be dismissed as of no practical importance. Therefore, the possibility of lint becoming spore-contaminated at the gin is very unlikely.

There still remains the possibility of the lint becoming infected from, or contaminated with, débris from the diseased plants themselves. Ginned seed usually has very little or no adhering débris, that most likely to be present being fragments of dried bracts. The fungus may invade the bracts, but how long it would remain alive in tiny, dry fragments of this tissue is unknown. Considering the comparative rapidity with which it dies in the woody stems, its chances for survival would seem to be slender.

In any case, the cotton fiber is a poor medium for the growth of the fungus. Only cotton wet to the point of saturation was attacked. Cotton, delivered to gins, ordinarily is sufficiently protected against rain or other moisture to preclude Verticillium infection of the fiber.

Herbert and Hubbard (6) harvested seed from diseased cotton plants and planted it without delinting it. The plants produced were healthy and normal in every way. The writers also repeated this experiment but were not certain that the ground in which they planted their seed was absolutely healthful. A small amount of disease developed under conditions that tended to confirm their suspicions that the soil was not sanitary at the outset.

Again, the writers do not wish to infer that transmission of Verticillium wilt by lint-bearing seed is wholly impossible, but rather that it is improbable. Among many other really important means of disseminating the disease, the use of such seed probably is of little or no importance.

Cotton-seed production in California is licensed. Production is confined as far as possible to healthy ground. Accordingly, the great preponderance of plants are free of Verticillium wilt and the seed produced is healthy. Even if some plants in the fields develop the wilt, the total amount of seed produced by them would be negligible in comparison to the total production. In the light of the experimental results obtained, the number of seed from such fields that could actually transmit Verticillium wilt is infinitesimally small.

Among the commoner, authentic and definitely proved means of disseminating the parasite may be mentioned the transfer of infective débris from one field to another on plows, tractors, etc. Cotton growers often transfer farm machinery long distances by truck, and little or no effort is made to remove coarser roots or other plant material clinging to it. The use of cuttings from diseased plants as well as the planting of infected nursery stock, including fruit trees and ornamentals, is one of the commonest methods of dispersal. Potato-seed tubers often are diseased, but may not be discolored. A common fallacy is the belief that all infected tubers have a brown discoloration of the vascular system or at least a brown spot at the point of stolon attachment. Most of them do, but certainly not all.

It has been stated that the disease has appeared suddenly in desert areas that had never been under cultivation. The inhabitants of such regions probably plant home gardens, shade trees, etc., and introduce the parasite with their plants and trees. In addition to stone fruits, bush fruits, truck crops, field crops, and ornamentals, *Verticillium albo-atrum* attacks many of our common weeds. It is not altogether impossible, therefore, that the disease may exist in weeds in some of the desert land cultivated for the first time. Reports of sudden "outbreaks" of the disease in virgin, desert soil are not wholly convincing. The average grower would not recognize or be concerned with the single, individual cotton plant that might develop the disease among the thousands in his field, assuming the disease to have been introduced on débris, etc. On the contrary, he would ignore it and eventually disk it into the soil along with the others at the end of the season, thereby establishing a real focus of infection the following year. The second year there might be enough disease present to constitute an "outbreak." The experiments and observations of Presley[7] in Arizona sustain the views expressed here. He states: "We have found that land in crop for the first time is often a veritable hot-bed of the disease and infections have appeared simultaneously in valleys which are widely separated and physiographically isolated. One of these areas known as Hidden Valley seemed to lend itself admirably to the study of *Verticillium* and its origin. The valley is well isolated from other cultivated areas, and pump water is used for irrigating the land. In 1937 Verticillium wilt was found there in a planting of Upland cotton, and though the disease was not severe several distinct spots were apparent. For three successive years additional land within a mile radius of the diseased field was brought under cultivation and planted to cotton as a first crop. Precautions were taken to obviate as far as possible any chance of seed-borne infection entering these plantings. The planting seed was acid-delinted at a commercial delinting plant in Mesa, Arizona, and one planting was made with seed of the D and P L variety that had been imported from an area known to be disease-free. In every instance the newly planted cotton showed, at various places in the field, typical wilt spots where the plants were killed outright or were severely stunted during early de-

[7] See footnote 6.

velopment producing bare spots in the field surrounded by plants which showed unmistakable symptoms of Verticillium hadromycosis."

SUMMARY

Cultures made over a 5-year period from 3371 mature cotton bolls produced on plants severely affected with Verticillium wilt showed that the fungus had succeeded in reaching only 150 receptacles (4.44 per cent), and had penetrated to the bases only of the placental columns of 2 bolls (0.00059 per cent). Cultures made from the seed produced by diseased plants showed it to be neither internally nor externally infected by *Verticillium*.

To test a theory that the lint on cotton seed might become contaminated with infective material existing in the ground as the pickers drag their bags over it, cultures were made from non-sterilized, lint-bearing seed taken from the bottoms of pickers' bags. The lint on such seed was dusty, but *Verticillium* never developed in the cultures. Similarly, to determine whether the parasite produces microsclerotia on dead cotton stalks left standing overwinter in a field, a total of 11,723 cultures were made during a 5-year period of the tiny, black bodies, erumpent or superficial, produced on such stalks. *Verticillium* was never found in the cultures. Also, to determine the longevity of the fungus in such old, dead stalks, a large number of cultures made over a period of years showed that the fungus dies out fairly rapidly in the tops after the plants have been killed by frost, but it remains alive until the following spring in many of the tap roots that have not decayed, as well as in the heavier woody portions of the stems at ground level. Lastly, to test a theory that the lint becomes infested by the fungus at the gins, cotton removed from bolls and steam-sterilized in culture tubes was inoculated with fragments of microsclerotial crusts of *Verticillium albo-atrum* produced on Czapeck's agar. The cotton constituted a poor medium; only lint wet practically to the point of saturation supported the growth of the fungus. It is exceedingly unlikely that cotton at the gins would become wet enough to enable *Verticillium* to attack it.

The experiments show that dissemination of Verticillium wilt by seed produced on plants affected by the disease is highly improbable. Also, that contamination or infection of the lint on healthy seed is equally improbable.

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INFECTION OF COTTON SEEDLINGS BY COLLETOTRICHUM GOSSYPII AS AFFECTED BY TEMPERATURE[1]

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Damping-off of cotton seedlings as caused by the anthracnose fungus was among the first of the seed-borne diseases to be studied intensively (1, 2, 3), yet there is little available information on the influence of specific environmental conditions on the incidence of infection by this fungus, except for the studies of Lehman (5). He studied the effect of 5 temperatures on the infection of seedlings which developed from seeds inoculated with spores of *Colletotrichum gossypii* South. Temperatures between 25° and 30° C. were most favorable for seedling infection. The data reported in this paper were secured in studies initiated to ascertain the extent to which Lehman's results might be applicable to naturally infested seed.

METHODS

Four lots of fuzzy cotton seed were germinated and the seedlings grown on 2 per cent agar gel, containing 0.002 *N* concentrations of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, and having an initial pH of 6.5. Approximately 6-cc. portions were placed in 18 × 150-mm. test tubes, which were then plugged and autoclaved. The usual irregular germination of fuzzy cotton seed on an agar gel was overcome by liquefying the gel at the time of seeding, cooling it in a water-bath to 45° C., and then dropping the seeds on the liquefied gel. A small but sufficient amount of the fuzz on the seeds became embedded in the gel to facilitate adequate water absorption for rapid and uniform germination. All data for a certain lot of seed at a given temperature are based on the germination of 100 seeds.

The seedlings were grown in light-proof chambers at a relative humidity of approximately 85 per cent and at several temperatures with maximal fluctuations of ½° C. The relative humidity within the plugged test tubes was not measured, but it probably was equal to or slightly exceeded that of the chambers. At the end of 10 days the seedlings, except those grown at 18°, were removed to the laboratory, where they were grown for an additional four days at temperatures which ranged from 22° to 28° C. The removal to the laboratory was desirable because of the great elongation of the hypocotyls when the seedlings were grown in the dark for a longer period at these temperatures. This change in conditions could not have greatly influenced the results; for the critical period for seedling infection at these temperatures was during the first 8 days. The concurrent germination of the same lots in the laboratory also indicated that the laboratory temperatures

[1] Contribution of the Department of Botany and Bacteriology in Cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Technical Contribution No. 90, South Carolina Agricultural Experiment Station.

were favorable for infection. On account of the slow growth of the seedlings at 18°, it was necessary to grow them in the cases for two weeks, after which they were observed in the laboratory for an additional week. When the hypocotyls had attained a length of 70–80 mm. and the cotyledons had come in contact with the cotton plugs, the latter were removed. The first seedlings reached this length in approximately 12 days at 18°, 7 days at 22°, 4 days at 25°, and 3 days at 29°, 33°, and 36°. At the first four temperatures, the number of days indicates the approximate time required for complete emergence of the cotyledons from the testas. At 33° and 36°, many of the cotyledons did not emerge until after the plugs were removed, and the seedlings had been placed in the laboratory. These two temperatures, especially 36°, are too high for the optimal development of the cotyledons. When the agar at the higher temperatures became reduced in volume as the result of water loss, an attempt was made to maintain a water level at approximately that of the original agar gel by adding, on alternate days, the necessary amounts of the same salt solution as that used for making up the agar gel.

The four lots of seed of upland cotton (*Gossypium hirsutum* L.) used were of the 1938 crop. The varieties, the states in which they were produced, and the letters by which they and their seedlings will be referred to in this paper are as follows: A. Mexican Big Boll, North Carolina; B. Dixie-Triumph, South Carolina; C. Stoneville 2b, Mississippi; D. Stoneville 2b, Georgia. The germination of the first three lots under favorable conditions was approximately 90 per cent; that of the D lot was generally from 10 to 15 per cent lower. All four lots were externally infested by the anthracnose fungus (*Colletotrichum gossypii*) and *Fusarium moniliforme*. Lots A, B, and C had from 2 to 5 per cent of the viable seed infected internally by *C. gossypii*. There was apparently no similar internal infection of the D lot, for there was no infection of seedlings which developed from surface sterilized seeds.

The development of a primary root and a hypocotyl of sufficient length to raise the testa or cotyledons above the surface of the agar was the criterion for determining the percentages of germination. Those seeds which developed only radicles were counted as not germinating. Healthy seedlings were those which germinated normally and had no visible lesions on the cotyledons, hypocotyls, or radicles. At least 25 per cent of all lesions were examined microscopically to verify the macroscopic observations. However, all lesions atypical of those produced by the anthracnose fungus were examined microscopically to assure that other fungi were not overlooked. A seedling was recorded as dead when the cotyledons became withered and dry or the hypocotyl had been so injured by the fungus that it was no longer able to support the cotyledons in an upright position.

RESULTS

In the initial experiments the B and C lots were grown at 18°, 22°, 25°, 29°, 33°, and 36° C. to ascertain the temperature at which the anthracnose

fungus would cause the greatest injury to the seedlings. The total germination of both lots was about the same at the various temperatures and ranged from 81 to 91 per cent, except for an emergence of 75 per cent for the B lot at 18°. The extent to which the low germination of lot B at 18° may have been associated with a high infection of the non-germinating seed by *F. moniliforme* is uncertain. The number of living seedlings per 100 seeds after 14 days for both lots was in the order $33° > 36° > 29° > 22° > 25°$. The number of healthy seedlings was in the same order, except for a slightly greater number of healthy seedlings at 36° than at 33°. Results for B lot are shown in figure 1. The number of seedlings infected but not killed at

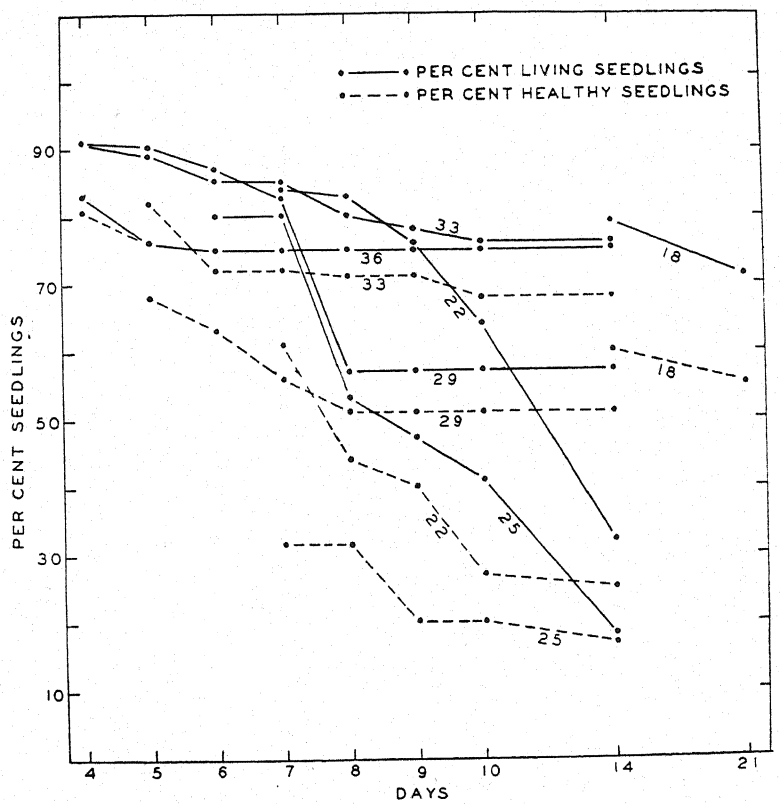


FIG. 1. The percentages of living and of healthy cotton seedlings of lot B (Dixie-Triumph) after incubation for the number of days indicated and at the temperatures denoted by the figures on the graphs.

each temperature is indicated by the difference between the solid line (percentage of living seedlings) and the dotted line (percentage of healthy seedlings). The first points to the left on the solid lines show the maximal percentages of germination; while the depression of these same lines to the right indicates the number of seedlings killed at successive intervals. The first points on the dotted lines were not recorded until the first visible lesions appeared on seedlings, so that healthy seedlings could be distinguished from infected ones.

Because of the much slower germination at 18° (a total of 85 per cent was attained on the 11th day) than at the higher temperatures, the results at the former temperature can be compared with the others only on the 14th day. At this time the percentage of living seedlings was largest at 18°, and the number of healthy seedlings for this temperature was midway between those at 29° and 33°. Relatively few of the seedlings which were alive after 14 days of incubation at 18° were infected and killed during the subsequent 7 days of incubation in the laboratory where the temperature was more favorable for infection. It is apparent that the reduction of the temperature from 22° to 18° decreases the susceptibility of cotton seedlings to injury by the anthracnose fungus, regardless of the slow growth of the seedlings at the lower temperature.

The number of seedlings infected at 33° and 36° (Fig. 2) was somewhat larger for the B lot than for the C lot during the first 7 days of incubation. After the 8th day the numbers for both lots at these temperatures were about the same. In contrast, at the lower temperatures the relative numbers of infected seedlings were approximately the same for the first day or two after the appearance of the lesions, but tended to differ after a longer period of incubation. Thus, on the 14th day the C lot had from 9 to 13 per cent more infected seedlings than the B lot at 22°, 25°, and 29°. There was a comparable difference between the lots at 18° C. on the 21st day.

A microscopic examination of the lesions showed that certain of the seedlings were infected by fungi other than *C. gossypii*. The anthracnose fungus did not infect the seedlings at 36° C., but 5 and 6 per cent of the cotyledons of the B and C lots, respectively, were completely rotted by a *Rhizopus* sp. within the first 7 days. The only other injury by *Rhizopus* was the destruction of approximately 4 per cent of the cotyledons of the seedlings of both lots at 33° and the destruction of one hypocotyl of the B lot at 18°. A *Penicillium* sp. was also found on 2 per cent of the severely injured cotyledons of the C lot at 36°. A *Rhizopus* sp., presumably the same as that infecting the cotyledons, was found on 25 to 50 per cent of non-germinating seeds of both lots at 18° and 36° and also on those of the B lot at 33°. The other temperatures apparently were not favorable for the development of this *Rhizopus* sp. or its growth was inhibited by the more rapid growth of other micro-organisms.

At 33° C. not more than 24 per cent of the seedlings of either lot were infected by the anthracnose fungus. The first lesions were visible on the 5th day, but few appeared after the 7th day; and all of the infected seedlings which died were killed before the end of the 10th day. About 40 per cent of the seedlings with lesions outgrew the injury. The seedlings killed by the fungus were generally those on which the lesions appeared earliest. In contrast, at the lower temperatures relatively few infected seedlings outgrew the lesions. At 29°, as at 33°, the first anthracnose lesions on the hypocotyls appeared on the 5th day. Most of these infected seedlings were killed before the 9th day, and only about 10 per cent of the seedlings infected at 29° outgrew the injury.

Although the anthracnose lesions appeared one to two days later at 22° and 25° than at 29° and 33° (Fig. 2), the greater number of seedlings were infected and killed at the two lower temperatures. At 22° and 25°, 80 and 87 per cent, respectively, of the seedlings of the C lot were infected; and 70 and 77 per cent, respectively, of the B-lot seedlings. Less than 10 per cent of infected seedlings were alive on the 14th day at both temperatures.

After it had been ascertained that the optimal temperature for the infection of the seedlings by the anthracnose fungus was approximately in the

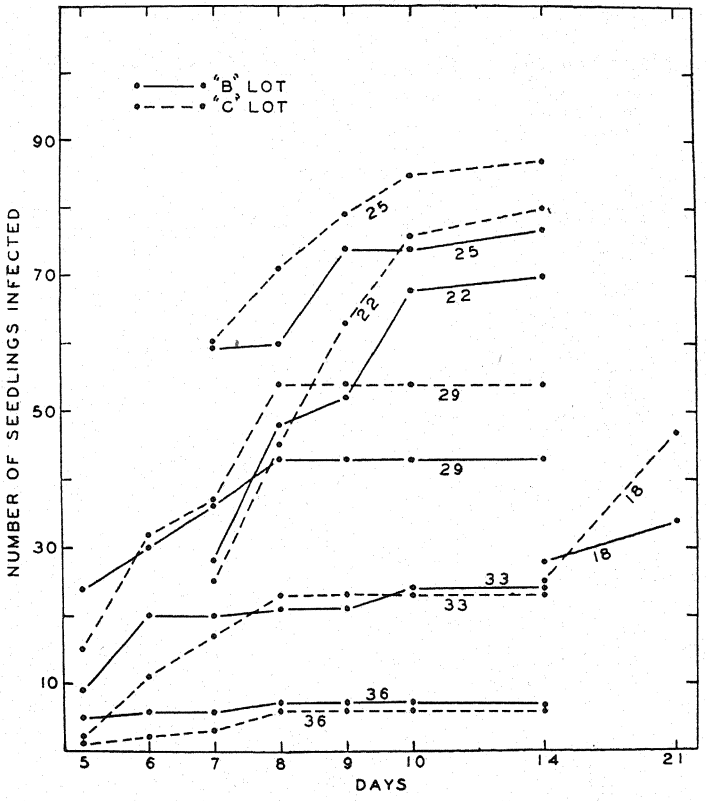


FIG. 2. The number of infected seedlings per 100 seeds of two lots of cotton seed ("B" and "C") after incubation for the number of days indicated and at the temperatures denoted by the figures on the graphs.

range of 22° to 25°, the four lots of seed were grown simultaneously at each of the two temperatures. At both temperatures the A lot gave the highest percentages of living and healthy seedlings (Fig. 3). At 25° the percentages of seedlings for the other 3 lots infected and killed were much the same, and the differences between them were small. At 22°, however, there were distinct differences among these 3 lots, the C lot producing the smallest number of living and healthy seedlings, the D lot the largest number, with the B lot intermediate. The proportion of healthy seedlings for the D lot was actually somewhat greater relative to the other two lots than is indicated on the graph on account of the 15 per cent lower germination of this lot.

The greatest difference in the incidence and severity of the lesions at 22° and 25° was shown by the A lot. At 22° after 14 days of incubation, there were 70 living and 63 healthy seedlings as compared to only 43 living and 38 healthy seedlings at 25°. Although the differences between these two temperatures for the other three lots were smaller, the graphs also show greater injury by the fungus at 25° than at 22°. This difference is further shown by the time required for the development of lesions. At 25°, there is a large increase in the number of infected seedlings during the 6th day with smaller increases for the next three days; while at 22°, there is a more

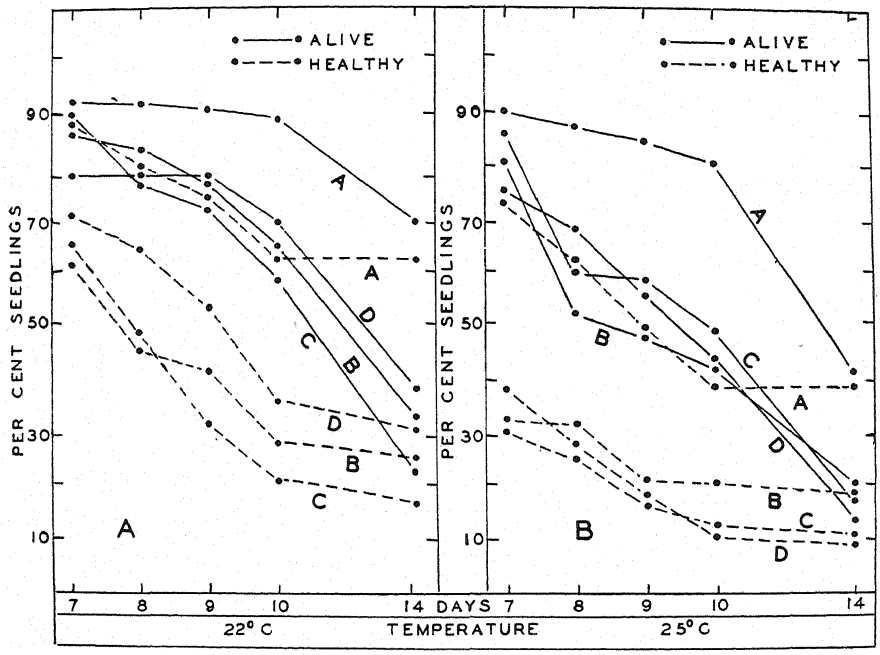


FIG. 3. The percentages of living and healthy seedlings of each of four lots of cotton seed after incubation for the number of days indicated at 22° C. (A) and at 25° C. (B).

gradual increase from the 7th to the 10th day. Much the same relative difference was shown in the time required to kill a given number of seedlings at these 2 temperatures.

Important differences were apparent among the four lots in the percentages of the seedlings which had anthracnose lesions on the cotyledons at the time when they emerged from the testas. The percentages for the A, B, C, and D lots at 25° were 40, 25, 25, and 0, respectively; while at 22°, they were 5, 10, 12, and 0, respectively. Most of the seedlings with lesions of this kind also had corresponding lesions on the hypocotyls. However, in the following instances about one-fourth of the seedlings with lesions on the cotyledons had no lesions on the hypocotyls: C lot at 22° and 25°, B lot at 22°, and A lot at 25°. The cotyledons of the A lot were especially susceptible to injury by the anthracnose fungus at 25° C.: 80 per cent of the infected cotyledons

were completely rotted before the 14th day. The percentages of cotyledons of the B and C lots rotted in this same period and at the same temperature were about one-half as great. The lesions which did not enlarge to involve the entire cotyledon mostly remained small and at the end of 14 days appeared as dry, brown spots 1–4 mm. in diameter. For the 3 lots in which anthracnose lesions appeared on the cotyledons, the lesions were generally larger as well as more numerous at 25° than at 22°. At the higher temperatures of 29° and 33°, less than 5 per cent of the cotyledons of the B and C lots were infected by the anthracnose fungus, and these lesions were invariably associated with corresponding lesions on the hypocotyls. At 18°, 5–6 per cent of the cotyledons of both lots were destroyed by *C. gossypii,* and 3 per cent by *F. moniliforme.* The destruction of cotyledons by a *Rhizopus* sp. and *Penicillium* sp. at 33° and 36° has been noted previously.

DISCUSSION

In 3 field plantings[2] in South Carolina in 1939 in which the same lots of seed were used, the surviving seedlings at 6 weeks after planting for the untreated seed of the A, C, D, and B lots were 53, 35, 28, and 25 per cent, respectively. The examination of the diseased seedlings showed that these differences were due largely to pre-emergence killing and damping-off caused by the anthracnose fungus. That the externally seed-borne anthracnose fungus was responsible for these differences in the number of surviving seedlings was also indicated by a comparison with a two-year-old lot of seed, "M," included in the field plantings. This lot was not infested by the anthracnose fungus and was of the same variety and strain as the A lot. Untreated seed of this two-year-old lot produced 68 per cent surviving seedlings, or 15 per cent more than A. The seed of the M, A, and C lots, after treatment with ethylmercuric phosphate, produced essentially the same percentage of seedlings, or 73, 73, and 67 per cent, respectively; while the percentages for the B and D lots were 60 and 46, respectively. Thus, treatment of the M, A, C, B, and D lots of seed increased the surviving seedlings by 7, 38, 91, 114, and 64 per cent, respectively.

The increases in seedlings in the field for the A and D lots approximated those which might have been expected from the percentages of their seedlings which were infected by the anthracnose fungus at 22° (Fig. 3). Similarly, the laboratory results would have indicated a greater response to seed treatment in the field for the C and B lots than for the A and D lots, but a greater response for B than for C would not have been expected. Thus, the germination of similar lots of seed at 22° by the laboratory method described should indicate their approximate response to seed treatment when planted in the field. Small differences among lots in the laboratory tests, however, may not be discernible in the field.

These results give no indication of the factors which may have been responsible for differences among the four lots. Ullstrup (6) has shown that

[2] These plantings were made in cooperation with the plant pathologists of 6 other states in a study of the effect of the characteristics of various seed lots on their response to seed treatment with ethylmercuric phosphate.

differences in pathogenicity exist among isolates of this fungus. Differences in spore loads, in degree of infection of the seed coat by the mycelia, and in innate resistance to infection by the cotton variety are not precluded.

The greater incidence of anthracnose lesions at 25° in these experiments than at 29° suggests that the optimal temperature range for seedling infection by *C. gossypii,* when naturally infested seeds are used, may be in the lower portion of the 25–30° range reported by Lehman (5) for inoculated seeds. His data on the growth of the fungus at various temperatures, however, coincide closely with the amount of infection at the several temperatures used in this experimentation; for temperatures of 25° to 30.5° were found by Lehman about equally favorable for the growth of *C. gossypii,* with the lower portion of this range the more favorable for conidial production. There was no growth of the fungus at 34.5°, and at 21° the growth was slower than at 25°. These temperature relations of the parasite seem to explain the absence of anthracnose infection of the seedlings at 36°, the low percentage of seedlings infected at 33°, the higher percentage infected at 29°, and the successively reduced infection at the two temperatures tested below 25° C.

Camp and Walker (3) and Lehman (5) supply comparable data for the germination and early growth of cotton seedlings. Their observations indicate that germination and seedling emergence is most rapid at 30° or slightly higher, is slightly less rapid at 25°, and is much slower at 20° C. A comparison of these data with the incidence of anthracnose lesions at the various temperatures indicates that the causal fungus has its maximal pathogenicity at a temperature, about 25°, which is near the lower limit of the temperature range for its maximal growth but is well below the optimum for the host.

The data by Walker (8) on the infection of onion by a closely related fungus, *Colletotrichum circinans,* indicate a different relation of temperature to disease production. The optimal temperature for disease production is 26–27°, while the fungus mycelium on agar grows best at 20–26°. This places the optimal temperature for disease production at the upper limit of the temperature range favorable for mycelial growth rather than in the lower portion of the range as for *C. gossypii.* The former parasite, however, is relatively more virulent at 31° than is the latter. This difference may be associated with the fact that temperatures above 30° are relatively more favorable for the rapid and normal growth of the cotton plant than they are for the onion.

Rhizoctonia solani, another fungus that causes damping-off of cotton seedlings (8), is most active at temperatures which are below the optimal for damping-off by the anthracnose fungus. The optimal soil temperature for the damping-off of cotton seedlings by *R. solani* is given as 17° to 23°, with little reduction in the number of seedlings killed until a temperature above 31.5° C. is reached. This *Rhizoctonia* also grows well over a wider range of temperature than does the anthracnose fungus. Field observations in South Carolina indicate that the rhizoctonia fungus is more active as a

parasite on cotton plants at temperatures below 20° than the anthracnose fungus; but that at higher temperatures their relative importance is reversed, especially when the planting of infested seed is followed by moderate temperatures associated with high soil moisture.

The rapid decay of a small percentage of the cotyledons by a *Rhizopus* sp. at 33° and 36° indicates that the causal fungus is most active at temperatures approximating those most favorable for *R. tritici* (9) rather than those for *R. nigricans*. No comparisons were made in culture of the various mycelia of the *Rhizopus* spp. found on the decayed cotyledons and on the non-germinating seeds at other temperatures.

SUMMARY

Cotton seed infested by *Colletotrichum gossypii* were germinated at 18°, 22°, 25°, 29°, 33°, and 36° C. Anthracnose lesions appeared earliest at 29° and 33°. Relatively small percentages of seedlings were infected and killed at 33°. At 29°, much larger percentages were infected and killed, but not so large as at 22° and 25°. The last temperature approximated the optimal temperature for maximal infection and injury to the hypocotyls and cotyledons. Reduction of the temperature to 18° greatly reduced the incidence of seedling infection. At 36°, seedlings were not infected by this fungus.

The temperature for the maximal damping-off of cotton seedlings by the anthracnose fungus was in the lower portion of the temperature range favorable for the growth of the fungus and below the optimal temperature range for seedling development. In these experiments, practically all seedlings infected at 25° and 22° C. were killed before the 14th day. At 33° many of the lesions remained small and did not greatly retard the growth of the seedlings. From 4 to 6 per cent of the cotyledons were destroyed by a *Rhizopus* sp. at 33° and 36°.

The differences among the four lots of seed as to the percentages of seedlings killed were somewhat greater at 22° than at 25° C. The percentages of the seedlings of the lots killed in the laboratory at 22° were indicative of the response of these lots to seed treatment in field plantings.

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TOBACCO DISEASE CONTROL BY CROP ROTATION[1]

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Rotation is one of the oldest methods of improving crop production, and agronomists have for many years carried on extensive field-plot studies to determine the rotation systems best adapted to particular crops and areas. It is recognized generally that rotation practices influence the incidence of plant disease, particularly soil-borne diseases; but it has not proven feasible to obtain much specific information on disease control from the usual rotation experiment, as Goss and Afanasiev (7) report. Pathologists have recommended rotation as a control measure for many diseases, but, lacking definite field data secured under controlled conditions, these recommendations have been based largely on studies of host range and field observations. It has been assumed that the reduction of diseases through crop rotations was essentially a problem of starving out disease-producing organisms by planting resistant or immune crops and by destroying susceptible weeds. The present writers used definitely laid-out field plots to evaluate rotation as a control measure for tobacco root knot (*Heterodera marioni* (Cornu) Goodey), bacterial or Granville wilt (*Bacterium solanacearum* E.F.S.), stem rot (*Sclerotium rolfsii* Sacc.), and Fusarium wilt (*Fusarium oxysporum* Schl. var. *nicotianae* Johnson). Work was initiated in 1926 in Georgia and has continued to date in that State, and also in North and South Carolina. All tests have been repeated at 2 or more locations. It is not the purpose of this paper to report in detail on these studies, or to recommend rotation practices for the specific diseases studied. Rather the writers propose to present some of the data to show that the problem of plant-disease control through crop rotation is extremely complex and that it is not safe to base recommendations on general assumptions.

The field areas used were first planted for one year to a susceptible crop to insure that infection was even and severe. The plots were of two types: (a) Inclosed plots, 12×24 feet, made of tightly joined creosote-treated boards that extended 8 inches below and the same above the ground surface. Units were separated by 4- to 6-foot borders of bare soil. (b) Field plots, of 1/40 to 1/5 acre in different series, laid out in the usual manner except that surface drainage was carefully controlled to prevent interplot washing. Precautions were taken in all cultural operations to avoid interplot movement of soil or crop remains. The inclosed plot series were set up primarily to eliminate all possible movement of disease between plots and hence to serve as a check on the larger field-plot series. The correspondence of disease

[1] Cooperative investigations of the Division of Tobacco Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, the Georgia Coastal Plain Experiment Station, the North Carolina Department of Agriculture, and the Agricultural Experiment Stations of North and South Carolina.

counts secured in inclosed and field-plot series was very satisfactory throughout. Disease counts were recorded on the Disease Index basis, a value of 100 indicating a maximum of disease and 0 no disease. Plot layouts were of the standard randomized-block type.

ROOT KNOT

Root knot affects numerous cultivated crops and weeds. Because tobacco is very susceptible, it serves as an excellent indicator crop and so provides a measure of root-knot-nematode populations in the soil. Rotation has been a standard recommendation for root-knot control, and the use of crops such as Brabham cowpeas and velvet beans has been suggested.

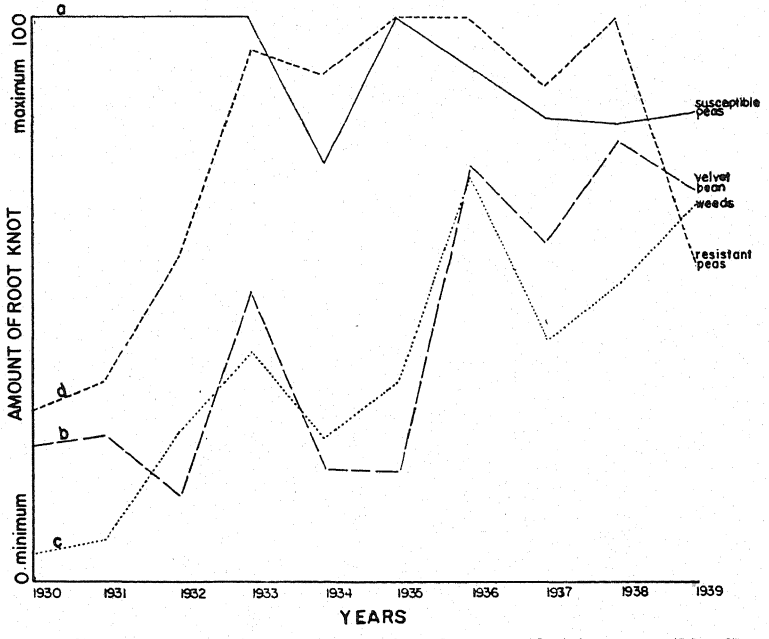


FIG. 1. Root knot on tobacco grown after 2 years of (a) susceptible Clay peas, (b) velvet beans, (c) native weeds, and (d) resistant Brabham peas. The results cover the period 1930 to 1939, inclusive, on land uniformly infested with root knot in 1926 at Tifton, Georgia.

Figure 1 shows the amount of root knot in flue-cured tobacco grown after 2 years of (a) susceptible Clay cowpeas, (d) resistant Brabham cowpeas, (b) velvet beans, and (c) native weeds over a 10-year period. During 1930 and 1931, tobacco after 2 years of resistant cowpeas (d), showed slight root knot, while tobacco after susceptible cowpeas (a) was severely affected. In 1933 this difference between susceptible and resistant peas disappeared, and throughout the remaining 7 years there was severe root knot on tobacco grown after both resistant and susceptible cowpeas. There was also a gradual increase in root knot on tobacco grown after velvet beans and after native weeds, with the result that the differences which were very striking

in 1930 were nonexistent or very slight in 1939. It might be assumed that resistant cowpeas, velvet beans, and weeds had become more and more susceptible to attack by root-knot nematodes, particularly in view of the recent important findings by Christie and Albin (2). Careful root examinations were made each year and there was no visible evidence that velvet beans and Brabham cowpeas were any more subject to root knot in 1939 than they had been in 1930. Both had slight traces of infection each year, but they otherwise were normal and healthy. Crops of susceptible cowpeas, however, were so severely affected by root knot that many of the plants died by midseason.

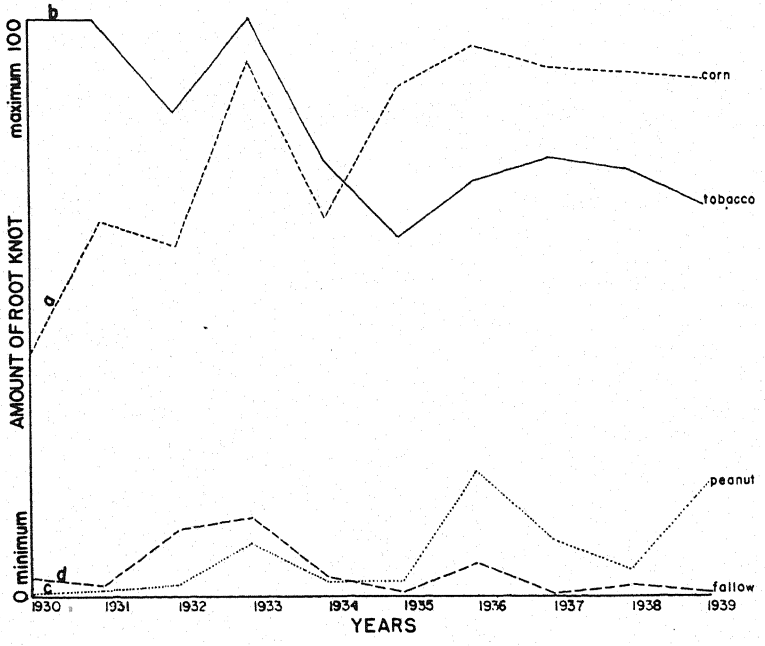


FIG. 2. Root knot on tobacco grown after 2 years of (a) corn, (b) tobacco, (c) peanuts, and (d) bare fallow, from 1930 to 1939, inclusive.

Corn also has been recommended for root-knot control (6), and under field conditions it is rare to observe galls on corn roots. Figure 2 shows the amount of root knot on tobacco grown after 2 years of corn, peanuts, and bare fallow, with a continuous tobacco check for comparison. Over the 10-year period corn was no better than continuous tobacco, and in repeated tests corn has never demonstrated any significant value in root-knot control. The disease actually decreased to some extent in continuous tobacco plots, while there was a definite increase in tobacco following resistant corn. The root-knot curves for tobacco after peanuts and bare fallow make a very sharp contrast with those for tobacco after the other crops. With bare fallow, starvation was highly effective in reducing root-knot-nematode popu-

lations, and the peanut crop was much more effective in starving out the nematodes than any of the others discussed.

In addition to the major crops grown during the summer, there are winter cover crops such as rye and oats that are highly resistant to nematodes, and others such as vetch and Austrian winter peas that are very susceptible. It would be expected that since the amount of root knot is reduced by bare fallow during the summer and generally increased by the culture of susceptible crops, the same principle would hold for winter practices. During

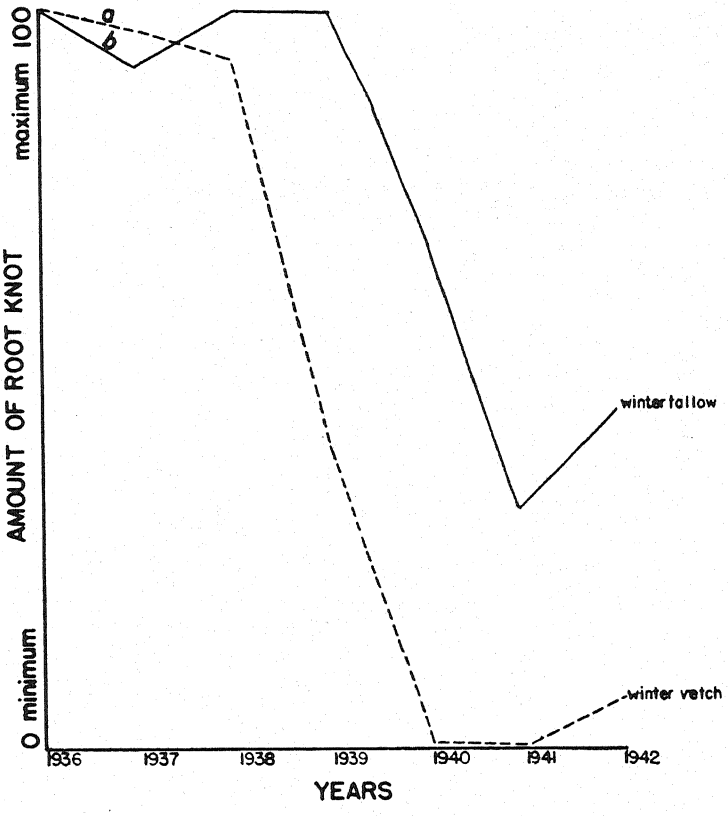


FIG. 3. Root knot in tobacco grown as a summer crop after (a) winter vetch and (b) winter fallow, from 1936 to 1942, inclusive.

the winter some plots were kept bare and some were seeded to winter vetch. The root knot in 7 successive summer crops of tobacco is shown in figure 3. In 1936–1938 root knot was very severe in all plots, but nematode population began to decline after the first 3 to 4 years. After 1938 there was much less root knot where tobacco was grown after a winter cover crop of vetch. During 1940–1942 tobacco grown after vetch was practically free of root knot, and the vetch, which had been severely galled during the early years, was also healthy. These findings are presented as indication that

culture of susceptible crops does not always increase soil nematode populations, and hence other very important factors are involved.

Additional basic problems associated with root-knot control by crop rotation can be illustrated with data from another experiment, at McCullers, N. C., laid out to provide maximum protection against interplot contamination. The units were 12 × 24 feet, and were tightly inclosed by board walls. Each treatment was replicated 4 times, and the mean values, with the appropriate error terms, are given in table 1.

TABLE 1.—*Influence of 2-year crop rotations on amount of root knot and yield of flue-cured tobacco, 1938 and 1940*

Tobacco grown after	Amount of disease[a]		Yield of tobacco (pounds green weight)	
	1938	1940	1938	1940
Crotalaria	38.8	42.8	43.4	70.5
Peanuts	41.0	37.3	31.0	46.8
Soy beans	84.8	59.0	40.4	58.4
Velvet beans	33.5	41.8	37.7	64.1
Sweet potato	100.0	95.8	34.6	40.4
Cotton	98.0	85.8	35.1	48.4
Corn	100.0	69.3	25.0	43.2
Weeds	84.5	83.3	31.3	47.4
Oats and weeds	86.3	86.5	33.7	45.6
Oats and fallow	35.3	35.5	30.8	43.3
Bare fallow	37.3	34.0	29.9	37.4
Tobacco	100.0	53.5	28.4	46.1
Difference required 5% level	8.76	17.45	5.14	4.31
for significance 1% level	11.77	23.44	6.90	5.79

[a] The amount of disease was recorded on the basis of 100 for maximum root knot and 25 for minimum root knot. (Each figure represents the mean value of 4 randomized replications.)

For all practical purposes a disease index value of 25 indicates complete freedom from root knot. The various crops were grown the first and third years and tobacco the second and fourth. One year of uniform tobacco cropping before the start aided in selecting an evenly and heavily infested area. The results in table 1 indicate that crotalaria, peanuts, velvet beans, oats plus bare fallow, and bare fallow were all very effective in reducing nematode infestation, and the differences in nematode control between these treatments were not significant. The weeds that followed oats were predominantly crab grass, and at this particular location small galls were observed on crab grass. It appears that crab grass following the oats nullified the effectiveness of oats, and the presence of crab grass in the weeds probably accounts for relatively poor results obtained with the weed rotation.

Practices giving good disease control and poor yields, however, would not be practical. Bare fallow has been suggested for nematode control and the data have shown it to be very effective; but in all experiments it has depressed yield. Tobacco grown after bare fallow was very free of root

knot but the yield usually was lower than that of more severely diseased tobacco following corn or even tobacco. Similarly, a healthy crop of tobacco was grown after oats followed by bare fallow, but the yield was less than with the severely diseased crop after oats and weeds. Bare fallow has reduced tobacco yield just as much as severe root knot, so that this treatment has not been a profitable one. Moreover, flue-cured tobacco quality was often lowered by bare fallow rotations, with the result that dollar returns per acre were still further reduced.

It is not unusual for fields with severe root-knot infection to produce excellent crops of tobacco, hence it is not safe to assume that a disease of this sort always reduces yield. Tobacco after sweet potatoes had severe root-knot infection in 1938 while tobacco after peanuts was practically free of root knot: the yield after sweet potatoes, however, was a little larger than after peanuts (Table 1). Root knot consequently is just one factor determining yield, and rotations giving maximum root-knot control would not necessarily be the most profitable for the grower to follow.

Assumption that failure to adopt the most stringent control measures will lead to constantly increasing damage does not appear to be justified in the case of a disease such as root knot. Thus, the experiment reported in table 1 was continued in 1942 and with an attack of only average severity there were no significant differences in the amount of tobacco root knot following the different rotations.

Despite the many complications, proper crop rotation remains by far the most practical and effective method of controlling root knot of tobacco. Control must be considered on a long-time basis, however, and the results from limited tests confined to a 3- or 4-year period can be quite misleading.

BACTERIAL WILT

Tobacco is very susceptible to bacterial wilt, and the list of host plants includes Irish potato, tomato, pepper, eggplant, peanut, and many weeds. Rotation has long been the main method of control recommended, but re-

TABLE 2.—*The occurrence of wilt-susceptible weeds in 1936 in relation to disease development in the subsequent tobacco crop in 1937*

Wilt-susceptible weeds per acre	Wilt in the tobacco crop	Wilt-susceptible weeds per acre	Wilt in the tobacco crop
Number	*Per cent*	*Number*	*Per cent*
0	27	6,758	28
40[a]	27	9,256	28
81	12	8,940[a]	19
162	36	74,792	3
324	36	116,278	44
728	27	140,024	11
761	5	389,976	35
1,011	35	574,312	35
1,492	10		

[a] Wilt-infected weeds were observed.

sults have been very uneven and failures to obtain control have frequently been attributed to growth of susceptible weeds in the immune crops. To test out this generally accepted theory weed counts were made in 17 separate wilt-infested fields in 1936 where growers expected to plant tobacco the following year. The results of the weed survey in 1936 and the percentage of wilt in the subsequent tobacco crops are given in table 2.

The range from 0 to 574,312 wilt-susceptible weeds per acre is very wide, but there was no correlation between the susceptible weed populations in

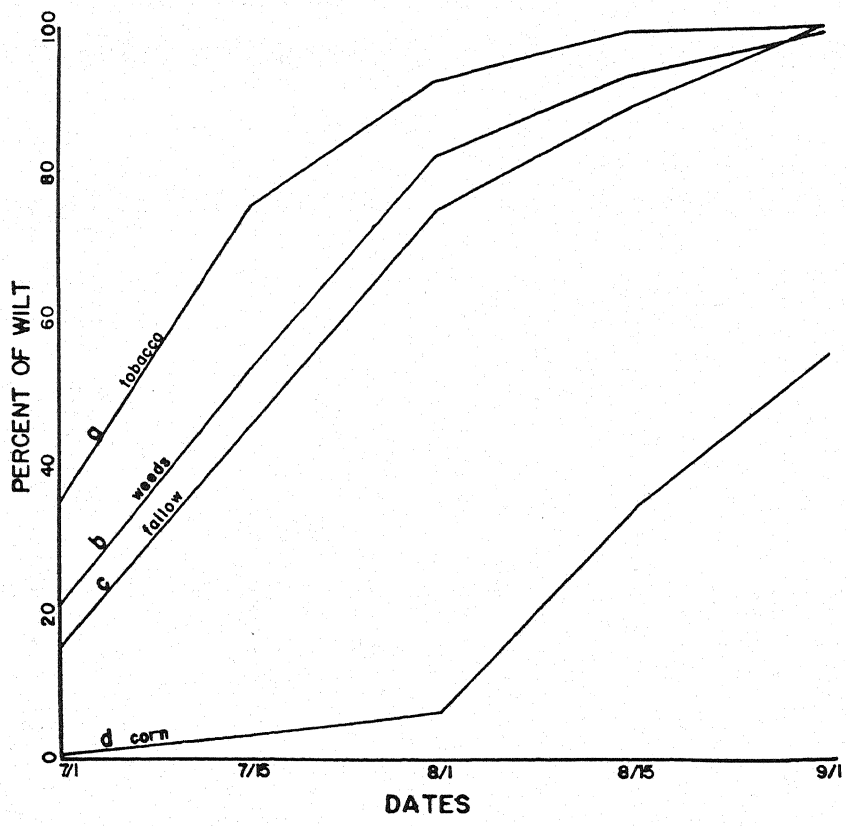


FIG. 4. The development of bacterial wilt in tobacco following (a) tobacco, (b) weeds, (c) bare fallow, and (d) corn, from July 1 through September 1.

1936 and the amount of wilt in the 1937 tobacco crop. Although many weeds are known to be wilt-susceptible, positively infected weeds are rarely observed under natural conditions. Wilt-infected weeds were observed in only 2 of the 17 fields in the survey. Further studies on the relation of weed host plants to the occurrence of wilt in tobacco were as follows: Plots were laid out in infested land and tobacco was grown after (a) corn and susceptible weeds, (b) weed-free corn, (c) crab grass and susceptible weeds, and (d) weed-free crab grass. Both corn and crab grass are immune to wilt.

Weeds in the crab grass grew poorly, but those in the corn grew vigorously and developed wilt following inoculation. These tests were repeated over a 4-year period and there was no difference in the amount of wilt developing in tobacco after weedy or weed-free preceding crops. It appears definitely established, therefore, that the failure of rotations with immune crops to give adequate wilt control in tobacco cannot be attributed to weed contami-

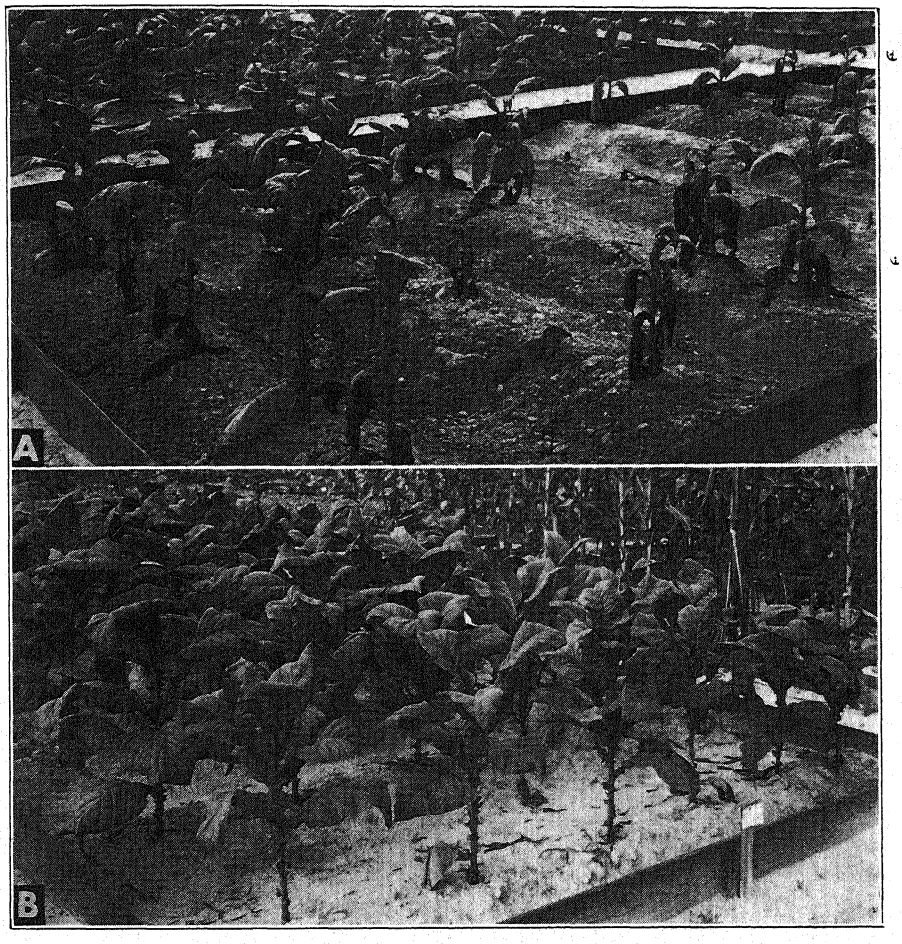


FIG. 5. A. Severe bacterial wilt in tobacco grown after bare fallow. B. The comparatively healthy tobacco produced after a year of corn.

nation. However, a natural weed fallow has been proven repeatedly to favor severe wilt in a succeeding crop of tobacco. The development of wilt in plots of tobacco grown after (a) tobacco, (b) natural weed fallow, (c) bare fallow, and (d) corn is illustrated in figure 4. The tobacco which followed a diseased crop of tobacco (a), was again severely diseased, as expected. However, tobacco after weeds (b) and bare fallow (c) was also severely affected. The general trend of these three curves is strikingly

similar, and on September 1 all these plots were 99 to 100 per cent affected. Corn greatly reduced the amount of wilt in the early summer. The heavy carry-over of wilt in the weed-fallow and bare-fallow plots cannot be explained on the basis of host plants, and the only possible explanation of the bare-fallow results is that this treatment provided conditions that favored persistence of the organism in the soil. Similarly the striking lack of infection after corn as compared with bare fallow (Fig. 4, c and d; Fig. 5) cannot be attributed solely to wilt immunity of corn.

STEM ROT

Root knot and wilt are caused by a nematode and bacterium, respectively, while stem rot is caused by the fungus *Sclerotium rolfsii* Sacc. Stem rot is widely distributed, but it is rare to observe a tobacco field with more than 5 per cent of the plants affected. Peanuts and soybeans, as well as tobacco, are attacked by stem rot, while crops such as corn and small grains are highly resistant. In the rotation plots at Tifton, Ga., tobacco-stem-rot counts have been made each year since 1926, when the plots were laid out, and similar records have been kept in other rotation series since established. The results have been consistent in that there was no relation between the character of the rotation and the amount of stem rot in tobacco. There was no more stem rot in tobacco grown after two years of stem-rot-susceptible peanuts than in tobacco grown after two years of resistant corn or after a complete bare fallow. At McCullers, N. C., where stem rot had been exceptionally severe on tobacco, in 1938, inclosed plots, 12×16 feet, were laid out in 1939 and rotations established with tobacco to follow tobacco, susceptible soybeans, and susceptible peanuts; also resistant corn and oats. All plots were heavily inoculated in 1939 and about 50 per cent of the tobacco, 15 per cent of the soybeans, and 70 per cent of the peanuts became infected. There was not a single case of stem rot in 1940 in the tobacco grown after these severely diseased crops, despite the fact that an abundance of sclerotia had been formed the previous year, an apparent confirmation of the statement by Wolf (15) that tobacco may remain free from stem rot even though the soil contains an abundance of the sclerotia. Although the 1940 tobacco had no stem rot, soybeans and peanuts grown after the 1939 diseased tobacco had, respectively, 40 and 32 per cent of stem rot in 1940. In 1941 there was again no stem rot in the tobacco plots, and the peanuts and soybeans had a trace to less than 5 per cent of disease. In 1942 the various continuous tobacco plots had up to a maximum of 14 per cent of stem rot, as compared with 0 to 8.7 per cent in the tobacco grown after other crops, but none of the differences were significant. The conclusion is that crop rotation had little effect on the amount of stem rot in tobacco, and that the use of such stem-rot-susceptible crops as peanuts and soybeans in tobacco rotations did not measurably increase the stem-rot hazard for later tobacco crops.

FUSARIUM WILT

Fusarium wilt is a comparatively new problem for tobacco growers of

the Southeast. At present it is important only in the Whiteville-Chadbourne area of North Carolina, but promises to become increasingly destructive unless growers take suitable precautions. Armstrong (1) recently showed that Burley tobacco is attacked by the same organism that causes Fusarium wilt of cotton. Smith and Shaw (13) found that certain physiologic races of the organism causing Fusarium wilt of sweet potatoes were able to attack both the green Orinoco varieties and the chlorotic Burley varieties of tobacco. The rotation experiments were not laid out with a view to studying the relation of crops to Fusarium-wilt occurrence but some very interesting observations were made. In four separate sets of rotation experiments, tobacco has been repeatedly grown after both sweet potatoes and cotton. In the Tifton, Ga., experiment, begun in 1926, Fusarium wilt occurred freely during the early years on both cotton and sweet potatoes. In 1937 it was first observed on tobacco, and in the sweet-potato rotation plots it has occurred in slightly increasing amounts each year since. Fusarium wilt has not yet occurred on the tobacco grown in the cotton rotation plots. At Florence, S. C., and other localities the rotation plots were initiated in 1936. Fusarium wilt has not been observed to date in the Florence experiment, but the disease was found on the Station farm where tobacco was being grown after a crop of sweet potatoes. At McCullers, N. C., Fusarium wilt was observed in tobacco the first year after sweet potatoes, and at Creedmoor, N. C., Fusarium wilt was observed in the 5th year of the sweet-potato-tobacco rotation. At none of these locations has Fusarium wilt been associated with cotton, or for that matter any crop other than sweet potatoes.

DISCUSSION

Of the four diseases studied, no two responded alike to crop rotations. There is considerable literature on root-knot control by rotation. Godfrey (6) concluded: "The most satisfactory method of combating the nematode in fields not planted to perennial crops is by the cultivation of immune crops for a period of two or three years and by carefully killing all weeds and susceptible plants in which the nematode can live. A desirable rotation is that where winter grains alternate with resistant cowpeas, Laredo soybeans, velvet beans, or beggarweed. Starving the nematodes by keeping the land free from all vegetation for two years is an effective control method, though often impractical." Le Roux and Stofberg (10), as the result of a 2-year test, concluded that nematode infestation could be effectively checked by eliminating all susceptible host plants. They also reported that bare fallow gave the best control and highest yields. Watson and Goff (14) found rotation the most practical method of control. They reported bare fallowing very effective, but discouraged the practice because of detrimental effects to the soil. In place of this, they recommended the use of root-knot-immune crops, particularly crotalaria and velvet beans. The writers have found root knot a good disease with which to study the comparative merits of various crop rotations. Previous workers have indicated preference for

crops thought to be completely immune to root knot, but the general practice has been to also recommend the use of crops regarded as moderately resistant, and no data have ever been given to show the comparative merits of the two groups. Figures 1 and 2 in the text bring out the fact that crops such as corn and resistant cowpeas, although they grew normally in heavily infested soils, did not starve out the nematodes, and actually were not superior to ordinary susceptible crops from the point of view of nematode control by crop rotation. Consequently, whether or not these resistant crops should be recommended for use on nematode-infested land depends on what the grower wishes to accomplish. If he desires to reduce the soil nematode population in preparation for growing tobacco or a susceptible truck crop, then his choice is limited to such apparently immune crops as peanuts, crotalaria, and oats. There were no significant differences between the reduction in nematode populations secured with these crops and the reduction secured with complete bare fallow. In all experiments, however, complete bare fallow reduced yields, and in one series bare fallow for merely a portion of the summer caused a greater yield reduction than the nematodes. Nematode control by bare fallow consequently would be neither practical nor profitable, particularly with light, sandy soils, where the nematode problem is most serious. So far as the writers are aware no one has called attention to the fact that under field conditions the nematode soil populations not only fluctuate from year to year but undergo gradual changes of great magnitude, even where the same cultural practices are followed many years. As would be expected, planting susceptible crops tended to build up maximum nematode populations, but with continued planting of susceptible crops the amount of root knot has not tended to remain constant. Maximum root-knot damage to plant roots in one series of continuous tobacco plots occurred during 1930 to 1933, and there was much less root knot in these same plots during 1935 to 1939. The corn-tobacco rotation plots in the same experiment showed an opposite trend, which would eliminate the possibility that these trends were associated with weather. Further evidence of the existence of important factors that influence root-knot-nematode soil populations and are entirely separate from crop susceptibility or resistance appeared in the experiments with tobacco following winter vetch or winter fallow. Despite the fact that vetch, as well as tobacco, is highly susceptible to root knot, the tobacco following vetch was practically free of root knot for the three years 1940 to 1942. Root knot decreased somewhat in the winter-fallow plots, but this tobacco continued to develop moderate to severe root knot. Linford, Yap, and Oliveira (11) have shown that it is possible to reduce nematode populations in the soil by the addition of organic matter, and their results and those of Drechsler (3) strongly suggest that nematode parasites may be an important factor in regulating nematode soil populations. The writers, however, have not obtained any marked nematode control by growing winter crops of rye, which under their conditions certainly added as much organic matter as the vetch. The note-

worthy difference was that in addition to organic matter the vetch built up a large reserve of nitrogen.

The results from the bacterial wilt rotation experiments were very different from those with root knot. Bare fallow, which was highly effective against root knot, was very ineffective against bacterial wilt. Tobacco planted after one year of complete bare fallow suffered almost as severely from wilt as tobacco grown immediately after a diseased crop of tobacco. On the other hand, the culture of corn for a year greatly reduced the amount of wilt in subsequent tobacco. Thus, immune-plant cropping and bare fallow, which gave equal and effective root-knot control, were not equally effective against bacterial wilt. The fact that many native weeds are susceptible to wilt has long been regarded as a complicating factor in controlling wilt by immune crop rotation. In actual tests, however, there was no correlation between the population of susceptible weeds and the development of wilt in subsequent tobacco. Furthermore, despite the fact that many weeds are known to be wilt-susceptible, it is rare to observe infection in natural weed growths on land known to be infested; and even when weeds were actually planted in crops of corn and permitted to become infected, the amount of wilt in the subsequent tobacco was not increased. It is assumed generally that allowing infested fields to "lay out" for several years fails to control wilt because some of the weeds are host plants. This assumption is open to question, and it may be that weed fallow merely favors survival of the organism in the soil. There is strong evidence that occurence and survival of *Bacterium solanacearum* in the soil depends to a large degree on factors other than culture of host plants. In the writers' experiments there was abundant infection remaining in soil after four years of complete bare fallow, and in general, immune crop rotations tended to greatly reduce but never eliminate the disease. Repeated culture of tobacco invariably increased wilt damage to tobacco, but Eddins (4) reports that in Florida, where the same disease affects potatoes, new fields are most severely affected and after several years of potato culture, wilt damage is greatly decreased. It would seem that control of bacterial wilt through crop rotation involves to only a limited degree the principle of "starving out" the organism. Certain crop rotation practices have been developed and used extensively in this country (5), and in the Dutch East Indies (9 and 12), which tend to greatly diminish the amount of wilt infection that carries over in the soil. A better understanding as to how such crops as corn tend to reduce wilt might make it possible to greatly improve our present rotation practices.

Despite the fact that *Sclerotium rolfsii* lives over in the soil from year to year, and that many common plants are susceptible to attack, such as peanuts and soybeans, while others, such as corn, are immune, extensive tests over a long period failed to show any connection between crop rotation and the occurrence of stem rot of tobacco. These negative results seem to indicate either that the organism is able to attack tobacco to only a limited de-

gree and under very special conditions, or that continuous culture of crops susceptible to *Sclerotium rolfsii* does not increase the amount of infestation in the soil and hence that parasitism is a minor factor in the life of this organism.

The results with Fusarium wilt seem especially to point to the great value of rotation plots set up for the study of disease control. Thus, recent work by Armstrong (1) and Smith and Shaw (13) clearly indicated the probability that the Fusaria causing wilt of either or both cotton or sweet potatoes might also attack tobacco. All 3 crops are extensively grown in the same areas of the Southeast. The rotation results support the conclusion that the culture of sweet potatoes and tobacco on the same land is hazardous and likely to lead to the establishment of a tobacco-Fusarium-wilt problem. Cotton can apparently be grown in the same rotation with tobacco of the Orinoco type without danger from Fusarium wilt.

CONCLUSIONS

Considering the general problem of disease control by crop rotation, these studies appear to justify the following conclusions:

The degree of disease control secured from crop rotation will vary considerably depending upon the year, the location, and the disease, as well as upon the nature and length of the rotation. A grower may rotate bacterial-wilt-infested land with the utmost care, and still suffer severe losses from wilt in occasional years, or in certain fields.

Resistant crops are not necessarily superior to susceptible crops as a means of reducing the carry-over of disease in the soil. Soil nematode populations were just as large after a crop of resistant corn as after a crop of susceptible tobacco. However, root knot was consistently reduced by the use of immune crops.

The occurrence of disease was generally related to the previous occurrence of host plants but the exceptions were important. There was as much stem rot in tobacco grown after stem-rot-immune corn as in tobacco grown after stem-rot-susceptible soybeans.

It was not true that complete elimination of all plants (bare fallow) favored most rapid elimination of all soil-borne parasites. Bacterial wilt infection survived better in the bare fallow plots than in plots which grew crop plants.

Rotations that affect the carry-over of soil-borne disease have other important effects on crop yields. Rotations favorable from the nutritional viewpoint may give high yields occasionally despite extensive disease development. Bare fallowing reduces root-knot-nematode populations but also depresses yields. Therefore, the figures for crop yield and the amount of disease are not always closely parallel, and rotations that are effective in disease control but reduce yields obviously have little value.

Rotation is the only practical method available for combating many serious diseases and the importance of developing rotation practices that

definitely aid in disease control is apparent. It is not possible to generalize with respect to rotation and disease control, for each disease presents its own special problem. The results from rotation experiments show wide fluctuations, hence data from isolated tests may be quite misleading. Reliable conclusions can be drawn only from controlled experiments under a variety of conditions and over a number of years. Considering the field of disease control as a whole, it appears that such data are conspicuously lacking.

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STEM RUST ON NEW WHEAT VARIETIES AND HYBRIDS[1]

HELEN HART

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Since 1925 increasing numbers of stem-rust-resistant wheats have been bred and distributed in the United States. It has been customary to grow plant populations in the field under both natural and artificial rust epidemics for several years before a variety is considered adequately tested for rust reactions. In recent years there has been a growing tendency to test seedlings of the most promising materials to known physiologic races of rust maintained in pure culture in the greenhouse. Thus it is possible to determine the reactions of the new lines to the physiologic races of rust prevalent during the years in which the new hybrid populations were developing before the wheat is ever released. In spite of thorough testing, previously unimportant or new physiologic races or biotypes of races may be especially virulent on the new lines. Within a few years these virulent races may increase to such an extent that the new wheats no longer resist rust when grown in the localities for which they were bred.

An outstanding example of the change in rust population occasioned by the use of a new wheat variety is that of race 56 of *Puccinia graminis tritici* and Ceres wheat (6, 15, 19). Ceres was distributed in 1926, and by 1934 it was grown on an estimated four and one-half million acres (2). In 1928 race 56 was first identified, and by 1934 that race made up 35 per cent of the stem-rust population (19). Race 56 continued to occupy first place among the stem-rust races identified in succeeding years (19) and was especially virulent on Ceres. In 1935 and 1937 race 56 was so destructive to Ceres that farmers in the eastern part of the spring-wheat area reduced acreages, and the extensive plantings of that variety were pushed westward to the drier parts of the spring-wheat area where rust epidemics were less frequent. Another example of the interrelations of hosts and rust races is to be found in *Puccinia graminis avenae* and oats. New oat varieties, with stem rust resistance from a Richland parent, are susceptible to race 8 of oat stem rust. Stakman and Loegering (16) call attention to the greater prevalence of race 8 in 1943 and point out the probable increasing importance of that race if the new oat varieties supplant those now grown.

ARTIFICIAL STEM-RUST EPIDEMICS IN THE FIELD

Between 1939 and 1943 many of the stem-rust-resistant wheat varieties and hybrids were grown experimentally at University Farm, St. Paul,

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Minnesota, to see if they would remain rust-resistant when subjected to variations in environment and to early and heavy rust inoculum. Nearly 40 varieties of wheat considered highly or moderately resistant to stem rust under normal growing conditions and a few of the standard rust-susceptible varieties were obtained at various times through the courtesy of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Division of Agronomy and Plant Genetics and the Division of Plant Pathology and Botany, Minnesota Agricultural Experiment Station. Pure cultures of 11 physiologic races of *Puccinia graminis tritici* were furnished by collaborators in the Division of Plant Pathology, Minnesota Agricultural Experiment Station and the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

Two sowings, sometimes three, were made each year, the first one as early as weather and soil conditions permitted. Sowings were duplicated in 1941 and 1943 and quadruplicated in 1939, 1940, and 1942. Numerous borders of stem-rust-susceptible Hard Federation wheat surrounded each plot of randomized rust-resistant varieties; and the borders served to establish and increase the stem-rust inoculum put there at regular intervals by means of hypodermic syringes. Experimental varieties never were inoculated directly, but none of the test varieties were more than 25 feet from the first rust infection centers in the borders; and by the time the borders were severely rusted no experimental plant was more than 5 feet from rust inoculum.

Most of the physiologic races of stem rust chosen for study were prevalent throughout the United States between 1930 and 1940 (17, 18, 19): races 10, 11, 15, 17, 21, 34, 36, 38, 49, 56, and 147. Race 19 was used also, but since Hard Federation is resistant to this race, there was not sufficient inoculum of it to spread to the experimental varieties. Biotype B of race 15 has been used since 1940 because it has been isolated from a number of rust collections throughout the United States and is virulent on Rival, on Vernal emmer, and on some of the Kenya wheats (8, 19). Race 147 was included because of its virulence on Vernal emmer, used in durum-wheat-breeding work by reason of its resistance to many of the other physiologic races of stem rust.

Pure cultures of each race were grown in the greenhouse during March and April, so that sufficient quantities of viable urediospores could be collected and stored in an icebox for a few weeks prior to field inoculations. Between May 20 and 27, as soon as Hard Federation plants had 3 or 4 leaves, groups of 3 culms throughout the borders were inoculated by hypodermic syringe with freshly prepared suspensions of urediospores in distilled water. Each race was suspended and inoculated separately and the clumps were tagged, so that it was possible to ascertain that all races developed about equally well and contributed approximately equal quantities of inoculum for spread to the experimental varieties. Within 7 to 10 days after the first

border inoculations, rust spores were produced in abundance and the rust-resistant varieties were exposed to inoculum fully 8 to 12 days earlier than the natural and less abundant stem-rust inoculum usually is blown into the St. Paul area. Border inoculations, two or three times each week, continued through June 25, as long as any of the Hard Federation plants remained in the boot and suitable for retaining the suspension of inoculum. Rust spread rapidly in the susceptible borders and to certain of the experimental varieties. Most of the experimental varieties, however, did not rust for 12 to 18 days after rust inoculum was spreading from primary infection centers in the border; and the time elapsing depended to great extent on the temperatures prevailing in early June. Low temperature delayed sporulation.

All varieties were examined frequently and whenever large uredia, indicating susceptibility, appeared on a variety, collections were made to determine which physiologic races were attacking the variety and which were potentially dangerous for it. Small, poorly developed pustules, indicating a more or less resistant reaction, were disregarded because they were not considered dangerous or particularly injurious under ordinary conditions.

STEM RUST IN FOUR WIDELY GROWN WHEAT VARIETIES

In 1939 three varieties of hard red spring wheat were grown over extensive acreages in the United States (1). Approximately three and one-quarter million acres were planted to Marquis (C.I. 3641), more than three and one-half million acres to Ceres (C.I. 6900), and about five and one-half million acres to Thatcher (C.I. 10003). Rival (C.I. 11708) was relatively new in 1939 and was planted on about 1,000 acres (1, 21), but since that time has become more important and is more widely grown in the spring wheat area.

Marquis has been susceptible to many of the physiologic races of *Puccinia graminis tritici* described (20) and usually is heavily rusted in the field unless inoculum is scarce and environmental conditions are very unfavorable for stem-rust development. Ceres remained moderately resistant to stem rust for a number of years after it was released; but it is particularly susceptible to race 56, so that since 1934, when race 56 surpassed all other races in prevalence throughout the Mississippi Valley (18), Ceres often has been as heavily rusted as Marquis in the field. Thatcher has been resistant or moderately resistant to many of the stem rust races that attack Marquis; consequently it had largely replaced Marquis and Ceres by 1939. Between 1934 and 1940 rust infection was light in Thatcher unless that variety was planted unseasonably late. Rival also is resistant to many of the physiologic races of stem rust, and prior to 1940 it was not heavily rusted in the field.

Table 1 gives the amounts of stem rust in, and the stem rust races identified in collections from, the four varieties grown at University Farm, St. Paul, Minnesota, in plots provided with abundant inoculum each year from

TABLE 1.—*The development of stem rust and the physiologic races responsible for stem rust in Marquis, Ceres, Thatcher, and Rival wheats from 1939 to 1943 inclusive*

Variety and C.I. No.	Percentage[a] of stem rust in each sowing and physiologic races[b] of stem rust identified in collections														
	1939			1940			1941			1942				1943		
	Seeding date		Rust races	Seeding date		Rust races	Seeding date		Rust races	Seeding date			Rust races	Seeding date		Rust races
	Apr. 27	May 19		Apr. 19	May 17		Apr. 25	May 16		Apr. 14	May 4	May 25		Apr. 20	May 4	
Marquis, 3641	60–90	70–85	34 56	80–90	75–90	11[2] 15[3] 36[3] 147[3] 17[4] 34[4] 56[5]	20–85	50–65	15 17 56	35–40	60–80	60–80	17 36[2] 147[2] 56[4] 34[6] 15[6]	55	40–65	11 34 56 15[2]
Ceres, 6900	40–70	50–80	34 56[3]	70–95	75–85	11 17 36 49 15[2] 147[2] 56[8]	40–65	35–55	10 21 34 36 15[3] 17[3] 56[4] 147[4]	12–35	30–60	50–80	34 147 17[3] 34[4] 56[4] 15[5]	15–55	15–40	15 147
Thatcher, 10003	5–12	10–30	15 34 36 38 56	15–45	30–65	11 34 56[2] 15[10]	Tr–8	Tr–5	10 36 56[2] 147[4] 15[5]	8–10	20–40	55–60	36[2] 15[11]	.....	Tr–30	15[2]
Rival, 11708	3–12	15–30	17 11[2] 34[2]	30–60	60–80	17 34 36 147 56[3] 15[6]	5–30	Tr–15	15 34 17[2] 56[2] 147[4]	15–20	30–50	55–70	17 56 147 36[2] 15[8]	.....	15–45	15[2]

[a] A range in percentage is given because there was considerable variation according to proximity to early inoculum.
[b] The exponent indicates the number of times a race was identified in collections from a variety.

1939 through 1943. Stem rust developed well on Marquis and Ceres every year, the percentages of infection varying with date of sowing, with proximity to early inoculum, and with environmental factors during a growing season. In 1940, 1942, and 1943, more stem rust developed on Thatcher and Rival under these conditions than had ever occurred before; and in 1940 and 1942 there was definitely more rust in late plantings of these two varieties than in early plantings. In 1940 there was 65 per cent stem rust on late-sown Thatcher and 80 per cent on Rival; and in 1942 there was only slightly less on late sowings of both varieties.

All four varieties were infected with several different physiologic races of stem rust, from 2 to 7 races being identified each year in collections from

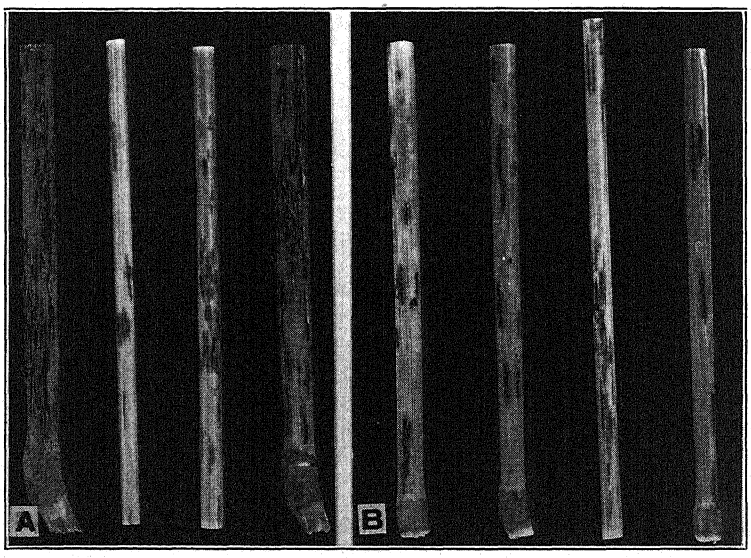


FIG. 1. The susceptible reactions of leaf sheaths and peduncles of (A) Rival and (B) Thatcher wheats to race 15 B of *Puccinia graminis tritici*. From field plots at University Farm, 1943.

a single variety. As reported previously (6, 15, 19), Ceres was an exceptionally good host for race 56, and in most years that race predominated in the rust collections from this variety. On Thatcher and Rival, however, race 15 predominated between 1940 and 1943; and in 1943 it was the only race obtained from these two hosts. Race 15 has a relatively wide host range, for it attacks 11 of the 12 varieties used as differential hosts for physiologic races of *Puccinia graminis tritici*, only Khapli emmer being resistant to it (20). Rival wheat acts as a differential host for biotypes of race 15, because it is resistant to biotype A and susceptible to biotype B (8). Biotype B of race 15 was first included in the inoculum in 1940, and in that year and subsequently it was a definite factor in causing rust on these four wheat varieties and many others in the experimental plots. Susceptible reactions occurred in Thatcher and Rival (Fig. 1) as well as in Marquis and Ceres,

all uredia on leaf sheaths and peduncles being well developed and without the signs of resistance that are so evident when Thatcher and Rival are attacked by less virulent rust races.

STEM RUST IN VARIETIES OF COMMON WHEAT CONSIDERED RESISTANT TO STEM RUST

A number of the new hybrid varieties that have been resistant to stem rust under farm field conditions were available in 1940 or 1941, among which were the ten varieties or selections listed in table 2. Of these, Hope probably has been the most resistant to stem rust over a period of years and while

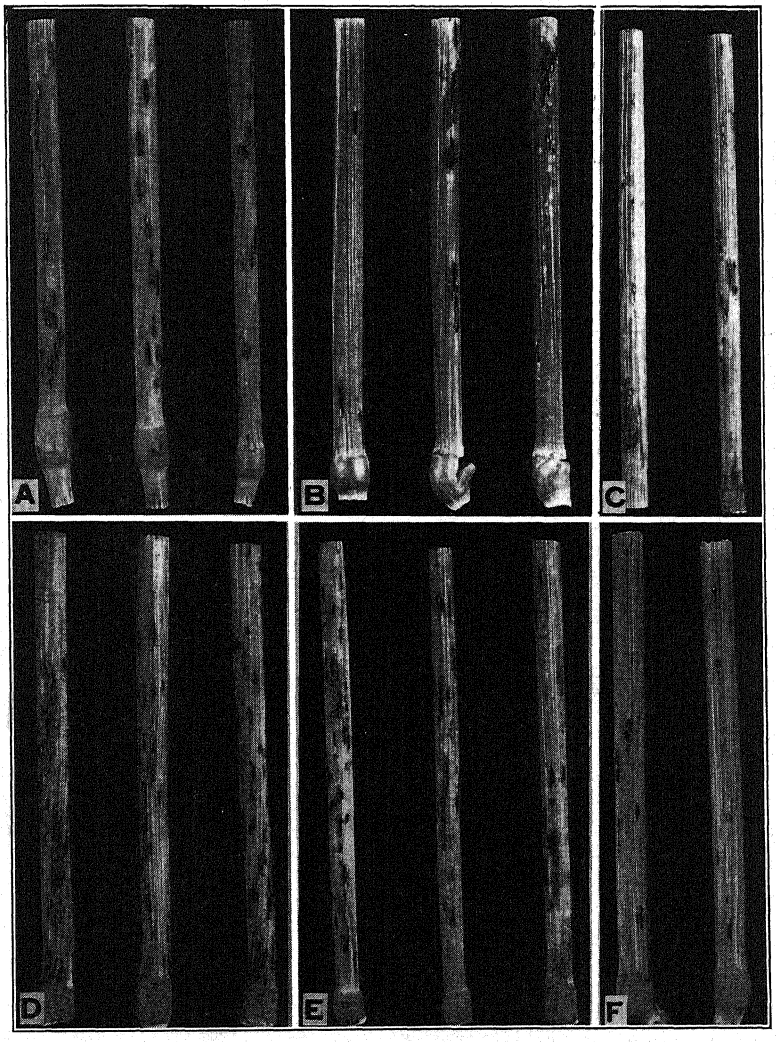


FIG. 2. Susceptibility to stem rust in (A) Apex, (B) McMurachy's Selection, (C) Renown Selection, (D) Premier, (E) Pilot 13, and (F) Regent Selection at University Farm, 1943.

TABLE 2.—*The development of stem rust and the physiologic races responsible for stem rust in varieties of common wheat usually considered resistant to stem rust*

Variety and C.I. No.	Percentage[a] of stem rust in each sowing and physiologic races[b] of stem rust identified											
	1940		1941			1942				1943		
	Seeding date	Rust races	Seeding date		Rust races	Seeding date			Rust races	Seeding date		Rust races
	Apr. 19		Apr. 25	May 16		Apr. 14	May 4	May 25		Apr. 20	May 4	
Apex, 11636	20–30	.....	10–30	2–15	147	10–20	30–40	50–60	15^2	15–20	18–30	15^2
Hope, 8178	Tr–20	.....	Tr	Tr	34	3–8	5–15	10–35	36^2 15^3 34^3	Tr–15	5–20	56 15^2 34^3
McMurachy's Sel., 11876	.....	.....	Tr–18	Tr–10	15 21 11^2 147^2	0–15	15–18	15–35	34 15^3	12–15	3–15	56 34^2 15^4
Mercury, 11872	15–30	.....	18–20	2–20	17	10–35	35–45	55–70	34 15^4	20–25	20–25	34 15^2
Merit 3, 12036	.....	.....	1–8	Tr–2	.....	Tr–3	15–30	50–60	15^3	5–15	5–15	15^2
Premier, 11940	5–20	15 56	2–12	Tr	17	8–15	35–40	20–60	15^3	3–30	5–18	15 34^4
Pilot 13, 11945	.....	.....	5–20	Tr–10	11 21	3–12	18–35	50–60	15^3	20–25	20–25	34 56 147 15^4
Renown Sel., 11947	15–25	15	10–25	Tr	.....	Tr–3	10–25	35–50	15^3	5–20	8–15	34 15^2
Regent Sel., 12070	2–25	.....	2–3	Tr	.....	Tr–2	5–25	15–35	56 15^2	Tr–10	Tr	34 15^2
Vesta, 11712	10–25	34	30–40	Tr–8	34	12–20	25–35	55–65	15^3	10–20	10–20	11 15

[a] A range in percentage is given because there was considerable variation according to proximity to early inoculum.
[b] The exponent indicates the number of times a race was identified in collections from a variety.

it has too many defects to be an acceptable commercial wheat it has been used extensively as rust-resistant parental material for breeding new wheats. McMurachy's Selection also has been considered an excellent source of stem-rust resistance (10, 13). Most of the others listed in table 2 were bred from crosses made between 1925 and 1935, and in some cases there has been re-selection for greater stem-rust resistance. All are considered moderately resistant to stem rust at present, but not all have been released or are recommended for commercial production. Apex, Renown, and Regent are grown commercially in Manitoba, Saskatchewan, and Alberta in Canada (10) and to a limited extent in the United States. Pilot has been grown on small acreages in the western part of the spring-wheat area of the United States (1, 21), and Vesta has been recommended for the western part of North Dakota (22).

The highest percentages of stem rust developed on varieties of this group in the late plantings of 1942. In that year even Hope, McMurachy's Selection, and Regent Selection had moderate rust infection and the other varieties were heavily rusted. Race 15 biotype B was responsible for most of the infection in 1942 and 1943, although race 34 was about as virulent as race 15 on Hope. In 1943 race 34 was the second most prevalent race according to identifications of rust from these varieties; and on Hope and Premier it was identified more often than race 15.

There were susceptible reactions in most of these varieties, for uredia were large and sporulated well and there was no chlorosis of tissues around the uredia (Fig. 2). Renown and Regent Selections were only moderately susceptible in the field, because many of the uredia were rather small although sporulation was good and there was no chlorosis (Fig. 2). Since race 15 B was identified in collections from all these varieties in the field plots, seedling reactions were determined in the greenhouse. No variety in this group was resistant to race 15 B: the infection type on seedlings of Premier and McMurachy's Selection was 3–, but it was 3++ to 4+ on other varieties.

STEM RUST ON RECENT HYBRIDS AMONG THE COMMON WHEATS

Multiple crossings have been made to combine desirable characters for quality and disease resistance of several promising wheats in one or more hybrids that will be satisfactory in all respects. Newthatch (C.I. 12328), which is now being released to farmers in Minnesota, was produced by crossing Hope and Thatcher and backcrossing the progeny to Thatcher twice. Several lines of similar hybrids exist, with varying degrees of resistance to both stem and leaf rusts. In other instances Thatcher or Double Cross have been crossed with H 44, the sister selection of Hope, or with Merit which originated from an H 44 × Ceres cross. Mida (C.I. 12008) was produced by crossing a selection from a Ceres-Hope-Florence combination with a selection from a Ceres-Double Cross combination. And in another case Reliance, Hope, and Comet have been used to produce a hybrid (C.I. 12050).

TABLE 3.—*The development of stem rust and the physiologic races responsible for stem rust in several complex hybrids among the common wheats*

Variety or hybrid and accession No.	Percentage[a] of stem rust in each sowing and physiologic races[b] of stem rust identified in collections from varieties									
	1941			1942				1943		
	Seeding date		Rust races	Seeding date			Rust races	Seeding date		Rust races
	Apr. 25	May 16		Apr. 14	May 4	May 25		Apr. 20	May 4	
Mida, C.I. 12008	.....	.....	.....	10–25	40–50	60–70	34 56 15^6	25–40	20–35	15^3
Reliance-Hope × Comet-Reliance-Hope, C.I. 12050	.....	.....	.....	18–25	40–55	65–70	.....	30	20–40	34 15^3
Newthatch, C.I. 12328	.....	.....	.....	.....	.....	.....	.....	20–25	10–18	34 36 15^5
Hope × Thatcher, C.I. 12199	2–3	Tr–5	.....	3–15	30–45	55–80	15^3	5–25	8–15	34 15
Hope × Thatcher, C.I. 12043	5–15	Tr	15	1–10	18–35	30–45	15^4	8–25	15–18	15
Hope × Thatcher, C.I. 12251	2–30	Tr–5	15	5–20	40	45–50	34 15^6	10–25	3–30	15
Hope × Thatcher, C.I. 12044	Tr–2	Tr–2	.....	Tr–5	15–35	30–40	15^4	10–20	10	34 15^2
D. C. × H44, II–29–52, Minn. 2681	Tr–3	Tr–6	.....	3–12	25–30	30–60	15^2	.....	.....	.....
D. C. × H44, II–28–27, Minn. 2682	5–40	Tr	15	Tr–8	.....	45–75	15	.....	.....	.....
Merit × Thatcher, Minn. 2705	.....	.....	.....	5–18	35–45	45–55	15^3	15–25	8–15	15^2

[a] A range in percentage is given because there was considerable variation according to proximity to early inoculum.
[b] The exponent indicates the number of times a race was identified in collections from a variety.

Many of these hybrids were supplied the author[2] and the results with a few of them are given in table 3. Most striking is the virulence of biotype B of race 15 and the preponderance of that race in all rust collections from

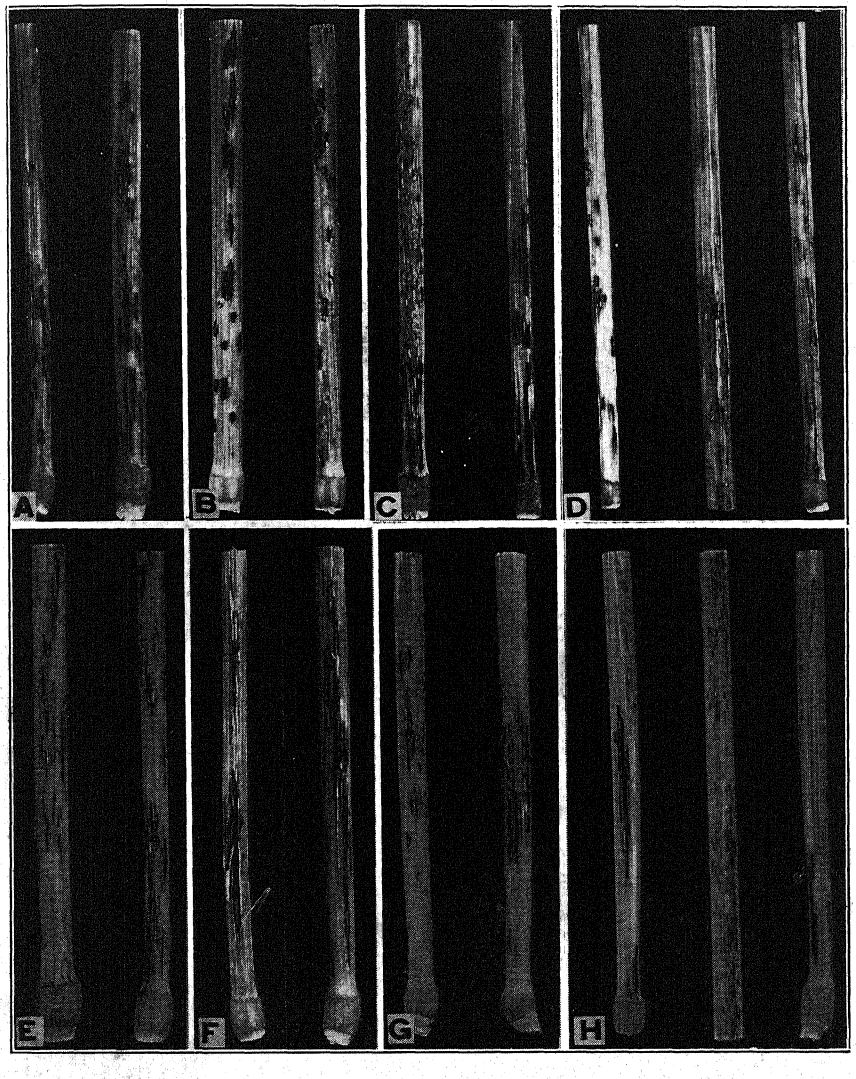


FIG. 3. Susceptibility to stem rust in (A) Merit × Thatcher hybrid (Minn. 2705); (B) a Reliance-Hope × Comet-Reliance-Hope hybrid (Minn. 2716 or C.I. 12050); (C) Mida; (D) Newthatch; (E) Iumillo; (F) Stewart; (G) a Mindum × Vernal hybrid (Minn. 2717); and (H) *Triticum timopheevi* at University Farm, 1943.

these hybrids from 1941 through 1943. The susceptible reactions of four of the hybrids from the field plots are shown in figure 3.

[2] Dr. E. A. Ausemus, Division of Cereal Crops and Diseases, United States Department of Agriculture, kindly furnished seed of these hybrids.

STEM RUST ON DURUM WHEATS, ON DURUM-EMMER HYBRIDS, AND ON TRITICUM TIMOPHEEVI

The durums Acme and Iumillo have considerable resistance to stem rust. Iumillo was crossed with the common wheat Marquis to obtain the stem-rust-resistant Marquillo (5); and Iumillo also went into the double cross (Marquis-Kanred × Marquis-Iumillo) from which Thatcher came (4). In years past both Acme and Iumillo have been lightly rusted in the field, but in 1942 both varieties were heavily rusted, especially in late plantings, and race 15 B was identified in all rust collections from the two wheats (Table 4). In 1943 the percentages of rust infection were not so high, but race 15 B again seemed to be responsible for a large part of the rust.

TABLE 4.—*The development of stem rust and the physiologic races responsible for stem rust in durum wheats, in Vernal emmer, and in Triticum timopheevi*

Variety or hybrid and C.I. No.	Percentage[a] of stem rust in each sowing and physiologic races[b] of rust identified						
	1942				1943		
	Seeding date			Rust races	Seeding date		Rust races
	Apr. 14	May 4	May 25		Apr. 20	May 4	
Acme, 5284	10–40	40–50	60–80	15^3	20–25	15–18	15^2
Iumillo, 1736	8–30	15–40	65–80	15^5	5–25	15–25	11 15^2 34^2
Mindum, 5296	10–30	55	65–75	.....	30–40	25–35	15 34^2
Vernal, 3686	Tr–20	30–40	35–55	.....	2–8	15–20	15^2
Carleton, 12064	15–30	60–70	60–70	15^5	25–30	20–30	15^3
Stewart, 12066	10–35	55–65	55–70	34 15^7	25–30	20	15^2
Mindum × Vernal, 12225	8–30	40–50	40–80	15^3	15–20	10–25	147 15^2
T. timopheevi, 11802	Tr–15	3–25	25–40	15^7	10–15	10–25	15^4

[a] A range in percentage is given because there was considerable variation according to proximity to early inoculum.

[b] The exponent indicates the number of times a race was identified in collections.

Rust-resistant durum wheats have been bred for the Red River Valley area of Minnesota and North Dakota and other sections of North and South Dakota by crossing the high-quality Mindum variety with the stem-rust-resistant Vernal emmer, then backcrossing the progeny to Mindum. Two new varieties, Carleton, C.I. 12064, and Stewart, C.I. 12066, have been named and released by the North Dakota Agricultural Experiment Station in cooperation with the Division of Cereal Crops and Diseases in the United States Department of Agriculture (14). They resemble the Mindum parent in growth habit and are late in maturing at St. Paul. In 1942 the parents and hybrids were heavily rusted in the author's experimental plots and biotype B of race 15 was prevalent (Table 4). Again in 1943, when stem rust was moderately heavy, race 15 B was more often isolated than any other

race. Both parents are susceptible to both known biotypes of race 15, so one would not expect the hybrids to be resistant. Whether biotype B may be more destructive to these wheats than biotype A over a period of years is not yet known, but all the hybrids had very susceptible reactions to stem rust in 1943 (Fig. 3), when race 15 B was prevalent in the experimental field plots.

Triticum timopheevi, considered highly resistant to stem and leaf rust and to several other diseases as well, was brought to the United States for

TABLE 5.—*The development of stem rust and the physiologic races responsible for stem rust in six Kenya wheats in 1943*

Kenya hybrid and accession No.[a]	Percentage[b] of stem rust in each sowing and physiologic races[c] of rust identified		
	1943		
	Seeding date		Rust races
	Apr. 20	May 4	
Kenya 122DI(I)(L), R.L. 1373, Minn. 2693	2–15	Tr–2	15^2 34^3
Kenya 117EB(I), R.L. 1374, Minn. 2694	15–30	15–18	15 34
Kenya 117B5B(E), R.L. 1375, Minn. 2695	15–20	10–25	56 15^2 34^2
Kenya 117K16A(L), R.L. 1376, Minn. 2697	25–35	10–35	56 15^2 34^2
Kenya 117I.5F.(L), R.L. 1377, Minn. 2696	8–30	15–25	34 15^2
Kenya 58	15–25	Tr–1	15 34^4

[a] The first designation for each wheat is that used by G. J. L. Burton of Kenya Colony, East Africa; the second is the number used by the Dominion Rust Laboratory, Winnipeg, Manitoba, Canada; and the third is the number used by the Minnesota Agricultural Experiment Station. Kenya 58 was obtained from M. N. Levine of the Division of Cereal Crops and Diseases, United States Department of Agriculture, who received it from R. J. Lathbury of Kenya Colony.

[b] A range in percentage is given because there was considerable variation in rust according to proximity to early inoculum.

[c] The exponent indicates the number of times a race was identified in collections from a variety.

breeding purposes, so that it might be crossed with other species of *Triticum* and contribute genes for its high degree of disease resistance to the progeny. The development of stem rust on *T. timopheevi* in 1942 and its moderate susceptibility to race 15 B has been reported by the author (3). In 1943 *T. timopheevi* was again rusted (Fig. 3), although not so heavily as in 1942; and race 15 B was the only race identified in four collections (Table 4).

Both Iumillo and *Triticum timopheevi* probably may be regarded as differential hosts for the two biotypes of race 15. In various experiments in Canada one or both of these wheats have been very resistant to race 15 (7,

11, 12), and it has been assumed that the Canadian race 15 was different from the race 15 B used in the present experiments. Seedlings of Iumillo inoculated in the greenhouse at St. Paul, Minnesota, had a 3++ infection type with race 15 B, while those of *T. timopheevi* had a 3+ infection type.

STEM RUST ON KENYA WHEATS IN 1943

Some of the wheats from Kenya Colony in East Africa have been reported very resistant to stem rust in North America (13) and in Australia (9). They are considered good breeding material in this respect. Six of the Kenya wheats were planted in the experimental plots in 1943. Nearly all were late maturing and were susceptible to leaf rust. Stem rust infection was not particularly heavy (Table 5), but some of the stem rust uredia

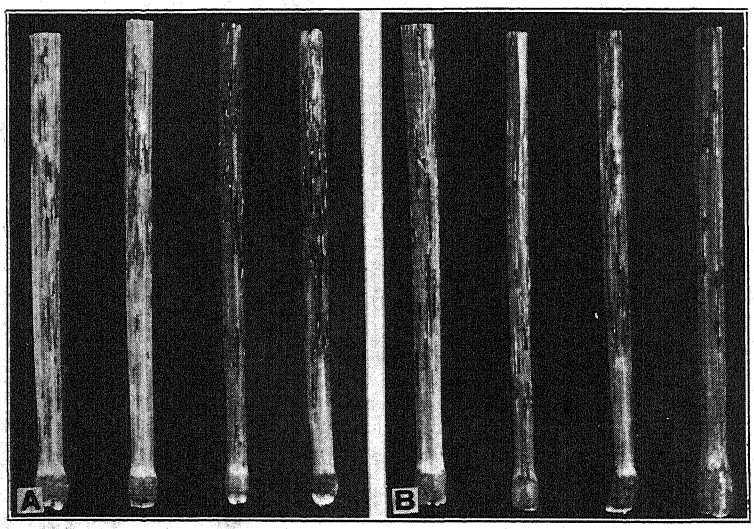


FIG. 4. Susceptibility to stem rust in (A) Kenya 117EB(I) and (B) Kenya 117K16A(L) at University Farm, 1943.

were large and sporulated so abundantly as to indicate a definite susceptibility (Fig. 4). Races 15 B and 34 were identified from rust collections from these wheats, and race 34 predominated. When race 15 B was identified, it usually was mixed with race 34 and constituted the smaller proportion of each collection. Peterson, Johnson, and Newton (13) found the Kenyas resistant to races 15 and 34 at ordinary temperatures and Watson, in genetical studies on the inheritance of the Kenya type of stem rust resistance, reported (23) his parental Kenya resistant to races 15 and 34. Later, however, Johnson and Newton (7) found that high temperatures made a Kenya selection, as well as some other wheats, susceptible to races 15, 29, and 56. They believe that temperature differences are responsible for many of the discrepancies in rust reactions of such wheats as the Kenyas at Winnipeg, Manitoba, at St. Paul, Minnesota, and at Manhattan, Kansas. Kenya seedlings were inoculated by the author with the biotypes of races 15 and 34

available at University Farm. Five of the Kenya hybrids were moderately susceptible to race 15 B, with 3 or 3+ infection types; while the hybrid Kenya 58 had 0; and 1 infection types and was resistant to race 15 B. When inoculated with race 34, seedlings of three of the Kenyas (Minn. 2695, Minn. 2696, and Minn. 2697) were moderately susceptible, with 3 or 3+ infection types; but seedlings of one Kenya (Minn. 2693) were resistant, with 1+ infection type. In one hybrid (Minn. 2694) some of the seedlings had 1++ infection types with race 34, while other seedlings in the same pots had 3+ infection types.

THE REACTIONS OF WINTER WHEAT HYBRIDS TO RACE 15 B

For the winter-wheat areas of the United States certain stem-rust-resistant hybrids have been produced by crossing a resistant parent such as Hope or Marquillo with winter wheats such as Oro, Tenmarq, Kawvale, or a Turkey selection. Seventeen such hybrids and 4 Kawvale × Tenmarq hybrids, together with the variety Pawnee (C.I. 11669), were sent to Minnesota for resistance studies.[3]

In 1943 vernalized seed of the 22 winter wheats were planted adjacent to the experimental plots of spring wheats. Thus the winter wheats were exposed to the rust inoculum provided for the other plots, and all 22 were well rusted by the time the plants had stooled. Since vernalization did not result in a general production of fruiting culms, stem rust on the leaf blades was collected for the determination of races attacking the hybrids. Race 15 B was identified in 14 of the 21 collections and race 34 occurred in 8 of the 21. Field reactions agreed very well with the greenhouse reactions of seedlings to these rust races. Seedlings of all were very susceptible to race 15 B, with infection type varying from 3+ to 4+; and all were moderately susceptible to race 34, infection types in that case varying from 3= to 4–.

DISCUSSION

Epidemics of wheat stem rust in the Mississippi Valley now are less frequent and less severe than formerly, because of the eradication of barberries, the changes in cropping systems, and the use of rust-resistant wheats. There is no assurance, however, that other severe outbreaks of stem rust may not occur in the future. The development of an epidemic depends on the presence of viable and virulent inoculum near the growing host and on the environmental factors that favor infection throughout the growing season. With eradication of barberries there has been a great decrease in the quantity of inoculum that once was produced rapidly and in abundance in many local areas. There probably has been a decrease in the diversity of inoculum as well, because many different physiologic races of stem rust may arise on the barberry through hybridization between existing races of *Puccinia graminis*. The growing of resistant varieties of wheat has reduced the

[3] Seed was supplied by Dr. K. S. Quisenberry, Division of Cereal Crops and Diseases, United States Department of Agriculture, cooperating with the Agricultural Experiment Stations of Kansas and Nebraska.

chances for rapid multiplication of inoculum and helped to change the population trends of many of the commonly occurring physiologic races of rust. The classic example of the effect of a new wheat on the rust population is the discovery and rapid increase in prevalence and destructiveness of race 56 following the release and extensive planting of Ceres wheat. Environment plays as great a part in the development of an epidemic of stem rust as does abundant virulent inoculum. Even with early and abundant inoculum of numerous virulent physiologic races of stem rust, the percentage of infection in a variety varies from season to season and even within a season. The year 1942 was exceptionally favorable for development of the artificial epidemic of stem rust, but even in that year conditions were less favorable for rust development early in the season than later.

Race 15 B of *Puccinia graminis tritici* was identified in several stem-rust collections from different parts of the United States, particularly near barberries. It is not one of the most prevalent races of stem rust but is potentially dangerous for the great majority of the wheat varieties and hybrids being bred for various parts of the Mississippi Valley. The varieties now grown in the spring wheat area also are susceptible to race 15 B, but on those hosts many different rust races are competing. Should there be a decided shift to some of the newer hybrids that are susceptible to race 15 B and resistant to the now prevalent races, there would be far less competition among races and more chance for an increase of race 15 B. Many of the breeding materials used as rust-resistant parents are moderately to highly susceptible to race 15 B. Among these are Hope and H 44, most of the Kenya wheats, Iumillo durum, Vernal emmer, and *Triticum timopheevi,* Relatively few wheats are highly resistant to race 15 B and most of these have certain undesirable characters. If biotype B of race 15 once becomes well established in the Mississippi Valley, which is not at all improbable, it may be very destructive to many of the new winter-wheat hybrids, to all the spring-wheat varieties now grown commercially, to most of the newer varieties and hybrids of hard red spring wheat, and to the new durum varieties. Susceptible hosts would be well distributed over the entire central and northern parts of the Mississippi Valley. With an increase in inoculum of race 15 B and with a wide distribution of susceptible wheats, it would be necessary to rely chiefly on environmental factors for the prevention of future stem-rust epidemics until resistance to race 15 B could be incorporated into other desirable wheats.

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INVESTIGATIONS ON THE TRANSMISSION OF BIG VEIN OF LETTUCE

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(Accepted for publication April 20, 1944)

Big vein of lettuce was first described by Jagger and Chandler.[3] They stated that the disease caused considerable loss to lettuce growers in the Imperial Valley of California. They advanced no theory as to the cause of the disease but reported that it was soil-borne and that partial sterilization with steam or formaldehyde would eliminate it. However, they presented no experimental data on soil transmission.

Big vein has been present in lettuce breeding stocks in the greenhouse and in field plots both at Beltsville, Md., and at Arlington Farm, Va., ever since the lettuce breeding work was begun at Arlington in 1929. Observations made on field trips to the principal lettuce-growing sections of the East during the past several years have indicated that big vein is widespread in the field.

Although the losses in breeding stock resulting from big vein have been small, it is always present in the greenhouse during the cooler parts of the year. Observations by Thompson and Doolittle[4] indicate that there is a temperature above which leaf symptoms are masked. The abnormal appearance of plants affected with the disease interferes with selection for type. Numerous tests of progenies from seed harvested from big-vein plants have yielded no evidence that the disease is seed-borne and its appearance in the greenhouse has suggested both soil transmission and dissemination by insects.

Since Jagger and Chandler reported on the disease in 1934, there have been no published data on the transmission of big vein. The present work began in the fall of 1942. It seemed possible that insect vectors might exist, so arrangements were made with the Bureau of Entomology and Plant Quarantine for cooperative studies and Floyd F. Smith of the Division of Truck Crop and Garden Insect Investigations studied possible insect vectors. Transmission through the soil and by mechanical means was studied by Ross C. Thompson and S. P. Doolittle of the Division of Fruit and Vegetable Crops and Diseases.

LACK OF TRANSMISSION BY MECHANICAL INOCULATION

Up to the present, all attempts to produce big-vein symptoms by mechani-

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[3] Jagger, I. C., and Norman Chandler. Big vein of lettuce. Phytopath. 24: 1253–1256. 1934.

[4] Thompson, R. C., and S. P. Doolittle. Influence of temperature on the expression of big vein symptoms in lettuce. Phytopath. 32: 542–544. 1942.

cal inoculation have been unsuccessful. Plants have been inoculated by leaf-rubbing with the use of carborundum powder, and by insertion of crushed tissue in incisions at the juncture of the petiole and stem. Roots were inoculated by dipping, as well as by cutting and crushing the roots while immersed, in juice extracts from big-vein-infected plants. Roots appearing at the opening in the bottom of the pots were inoculated by crushing and also by pricking the juice extract into larger roots. Other inoculations were made by injuring the roots with a spatula driven into the soil during the process. Crushed leaf tissue of big-vein-infected plants also was placed in the soil directly below the roots.

A total of 136 plants were inoculated by one or another of these methods. The inoculated plants were all held for 50 to 60 days at temperatures favorable to the appearance of the disease but no evidence of big vein occurred.

SOIL TRANSMISSION

During the winter of 1941 nearly every lettuce plant in about 300 ten-inch pots in the greenhouse had the typical symptoms of big vein. After the seed from these plants was harvested the soil was removed from the pots and after mixing, was stored in open bins in the field. This soil was used in tests during the winter of 1942–43, to determine whether big vein could be transmitted by this soil and, if so, whether steam sterilization would inactivate the causal agent.

A quantity of this soil that had previously grown big-vein plants was brought to the greenhouse in the late fall of 1942. It was divided, and one-half was steamed for three hours at approximately ten pounds pressure. The other half was left untreated.

In each of 3 tests on the soil transmission of big vein, 50 six-inch pots were filled with untreated soil and 50 with steamed soil. Lettuce sown in the two kinds of soil was transplanted to the respective soils, one plant in each pot. The pots were set on clay dishes to prevent possible contamination of the potted soil from the soil in the benches.

The 3 series were set up and handled in the same way, except that in series 3, the original untreated soil was not used. This series was started December 29, 1942, on the day that series 1 terminated. Soil from the untreated pots in series 1, in all of which big vein had appeared, was used for the untreated part of series 3. This soil was removed from the pots, mixed thoroughly, passed through a $\frac{1}{4}$-inch mesh screen to remove most of the lettuce roots, and returned to the pots. In other respects the 3 series were comparable except for date of planting.

A summary of the data from the 3 tests is in table 1. The data indicate clearly that big vein of lettuce is soil-transmitted and that the causal agent can be inactivated by steam treatment as stated by Jagger and Chandler.

From 38 to 51 days were required for the first plants to show symptoms of big vein, and a much longer time (88 to 127 days) for some of the plants to develop symptoms. Were it not for the fact that all efforts to transmit

TABLE 1.—*Summarized data from 3 tests on soil transmission of big vein of lettuce, in 1942–1943. Fifty plants in each series, in each soil*

Series I. Started Oct. 2			Series II. Started Nov. 2			Series III. Started Dec. 29		
No. plants showing big vein			No. plants showing big vein			No. plants showing big vein		
Date observed	Sterilized soil	Unsterilized soil	Date observed	Sterilized soil	Unsterilized soil	Date observed	Sterilized soil	Unsterilized soil
Nov. 16	0	1	Dec. 10	0	2	Jan. 22	0	1
Nov. 30	0	6	Dec. 26	0	15	Feb. 5	0	17
Dec. 7	0	10	Jan. 11	0	27	Feb. 15	0	34
Dec. 14	0	21	Jan. 30	1	33	Feb. 27	0	42
Dec. 21	0	36	Feb. 11	0	41	Mar. 8	0	46
Dec. 29	0	50	Mar. 9	0	48	Mar. 15	0	50
Total	0	50		1	48		0	50

the disease by mechanical means were negative, that no root aphids were present in any of the 3 series, and that the plants were kept free of other insects by regular and frequent fumigation of the greenhouse, it might be thought that the disease had been transmitted by some other agent than the soil.

Pryor[5] found that a relatively long time elapsed before big-vein symptoms appeared in his cultures and that some plants required more than 120 days in infested soil before symptoms became evident.

It should also be noted that symptoms did not develop in less time in series 3, in which the soil was used immediately after the removal of big-vein plants, than in the other two series. It is evident that the causal agent may persist for at least one year under field conditions. Further investigations of its longevity in the soil are in progress.

INSECT TRANSMISSION

Some preliminary experiments during the winter of 1941–42 indicated that certain insects might be vectors for big vein. However, the results of

TABLE 2.—*Transmission of big vein of lettuce by insects*

Insect vector	No. insects used	No. trials	No. plants infected[a]	Minimum incubation period,[b] in days
Aphis gossypii	250	1	0/10	.....
Macrosiphum solanifolii	175	2	0/10	.....
Macrosiphum n. sp.[c]	475	4	2/20	33
Myzus circumflexus	325	2	1/15	69
M. persicae	620	5	2/26	43
Trialeurodes vaporaorium	400	2	0/20	.....

[a] Number of plants infected over number exposed.
[b] Days until symptoms appeared.
[c] An undescribed species deposited in collection of Division of Insect Identification, Bureau of Entomology and Plant Quarantine, under T. C. No. 7335.

[5] Pryor, Dean E. The big vein disease of lettuce in relation to soil moisture. Jour. Agr. Res. [U. S.] 68: 1–9. 1944.

these experiments were variable and since adequate precautions had not been taken to prevent infection due to penetration of the roots into unsterilized soil in the bench beneath the pots, the data were considered inconclusive and are not presented. In all later experiments the pots were set on sterilized clay dishes to prevent contamination. After exposure to infective insects the plants were sprayed with a pyrethrum preparation and held in an insect-free greenhouse not previously used for growing lettuce.

The results of tests with five species of aphids and the greenhouse white fly, *Trialeurodes vaporaorium* Westw., are in table 2. No infections occurred in the plants exposed to white flies, to *Aphis gossypii* Glover, or to *Macrosiphum solanifolii* Ashm.; and only one or two infections occurred in the plants exposed to *Macrosiphum* n. sp., *Myzus circumflexus* Buckt., and *M. persicae* Sulz. In these experiments all species of sucking insects found on foliage of lettuce in local greenhouses were used. These included several vectors of a number of other viruses. Methods found successful for the transmission of both persistent and non-persistent viruses were used in these tests.

In later experiments during the spring of 1943, big-vein infection occurred in 8 of 10 plants following a transfer of 15 individual root aphids, *Pemphigus lactucae* (Fitch), from roots of a lettuce plant with symptoms of big vein. The aphids were transferred with a camel's hair brush, free of visible soil particles, to root-tips of healthy lettuce plants through the openings in the bottoms of the pots. The aphids increased rapidly after the transfer.

Uninoculated check plants and groups of plants that had been exposed to aphids and white flies from leaves of big-vein-infected plants were growing in the same greenhouse. After exposure the uninoculated plants were fumigated to eliminate these insects. At the time of the final examination, big-vein symptoms were present in 10 of the 60 uninoculated check plants and in from 40 to 70 per cent of the plants that had been exposed to *Trialeurodes vaporaorium, Myzus persicae,* or *Macrosiphum* n. sp. At this time it was found that the subterranean root aphids, which were unaffected by the fumigations, had become established on the roots of many plants in the above-mentioned groups. The natural spread of these root aphids from the originally inoculated to the other groups of plants seems to be the only logical explanation for the infection occurring in the unexposed checks. The same explanation seemed to hold for the greater portion, if not all, of those exposed to the other aphids and white flies, in view of their previous inefficient performance (Table 2). According to Cutright,[6] the young of *Pemphigus lactucae* depart from the usual behavior of young aphids. Instead of settling about the mother, the newly born microscopic young actively migrate among the soil particles or on the surface for 100 feet or more in search of food.

Investigations on insect transmission of big-vein have been discontinued during the present emergency period. Any further investigations to con-

[6] Cutright, C. R. Subterranean aphids of Ohio. Ohio Agr. Expt. Sta., Bul. 387, pp. 175–238. 1925.

firm the evidence indicating *Pemphigus lactucae* to be a vector of big vein should aim to determine whether the virus is carried internally by the insect and spread by its feeding activities, or merely by external contamination through contact with infected soil. The strong migratory habits of this aphid and its general occurrence on many hosts would tend to increase its importance in the spread of big vein in the field.

SUMMARY

The data presented indicate conclusively that the big-vein disease of lettuce is soil-borne and that its causal agent can be inactivated by steam treatment of the soil.

Under the conditions of these experiments, the symptoms of big vein did not appear in the leaves of any of the lettuce plants until they had grown in infected soil for 5 weeks, and a much longer time (up to 127 days) elapsed before symptoms were observed in some plants.

A total of 136 plants were mechanically inoculated in roots, leaves, and stems, using methods which have been effective in transmitting virus diseases in the case of other hosts. Although the inoculated lettuce plants were held from 50 to 60 days at temperatures favorable for the occurrence of big vein, no symptoms appeared.

The experiments on insect transmission were not extensive enough to justify definite conclusions. However, the results indicate that the root aphid *Pemphigus lactucae* (Fitch) may be a vector for big vein.

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STUDIES OF DODDER TRANSMISSION OF PLANT VIRUSES

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INTRODUCTION

Transmission of the virus of cucumber mosaic by two species of dodder, *Cuscuta subinclusa* Dur. and Hilg. and *C. californica* Choisy, and transmission of curly-top virus by *C. subinclusa* was reported (2) in 1940.

Johnson (8) in 1941 reported the transmission of the viruses of aster yellows, sugar-beet curly top, pea streak, tobacco mosaic, and cucumber mosaic by *Cuscuta campestris* Yuncker.

Kunkel (10, 11, 12, 13), using *Cuscuta campestris,* transmitted the virus of cranberry false blossom to tomato (*Lycopersicon esculentum* Mill.), periwinkles (*Vinca rosea* L.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and *Nicotiana glutinosa* L.; the virus of peach rosette to tomato; and the virus of potato witches'-broom to sugar beet (*Beta vulgaris* L.).

Transmission of dodder latent-mosaic virus by means of *Cuscuta subinclusa, C. campestris,* and *C. californica* was reported by Bennett (3); and Bennett and Munck (4) obtained transmission of the virus of yellow wilt of sugar beet by means of *Cuscuta subinclusa* and *C. campestris.*

It was obvious from the time of discovery of transmission of viruses by dodder that this method of transmission offered possibilities for study of plant virus diseases, especially in connection with determination of the host range of the viruses concerned, not afforded by any other method of attack. Efforts were made, therefore, to compare the efficiency of three species of dodder in the transmission of several viruses representative of recognized virus types. The objective of these experiments was to determine the relationship of different types of viruses to a range of dodder species and to obtain information on the factors involved in transmission.

MATERIALS AND METHODS

Three species of dodder, *Cuscuta subinclusa, C. campestris,* and *C. californica,* were studied. The first is characterized by its coarse stems. Several species of green plants, mainly shrubs, growing in low places in southern California, are hosts. The second occurs on alfalfa (*Medicago sativa* L.) and other legumes, and the third is abundant on a number of desert plants of southern California. All three species grow well in the greenhouse and can be colonized on a wide range of experimental plants.

Viruses Tested

Sugar-Beet Curly Top. Several strains of the virus were tested, but a

[1] Indebtedness is acknowledged to Katherine Esau, University of California, College of Agriculture, for the information contained in this paper on the cytology and histology of the haustorium of *Cuscuta subinclusa* and also for figure 3.

strain known to produce severe symptoms on sugar beet was used for the results recorded.

Sugar-Beet Mosaic. The virus used was that which occurs commonly on sugar beets in the vicinity of Riverside, Calif. No strains of this virus have been recognized.

Sugar-Beet Yellow Vein. This virus has not been described but it has occurred each season since 1936 in experimental plots of sugar beets near Riverside, Calif., and is known to occur also in Colorado. It causes distinct yellowing along the main veins of the leaves, dwarfing of the plant (often more marked on one side), and general yellowing of the foliage. It is trans-

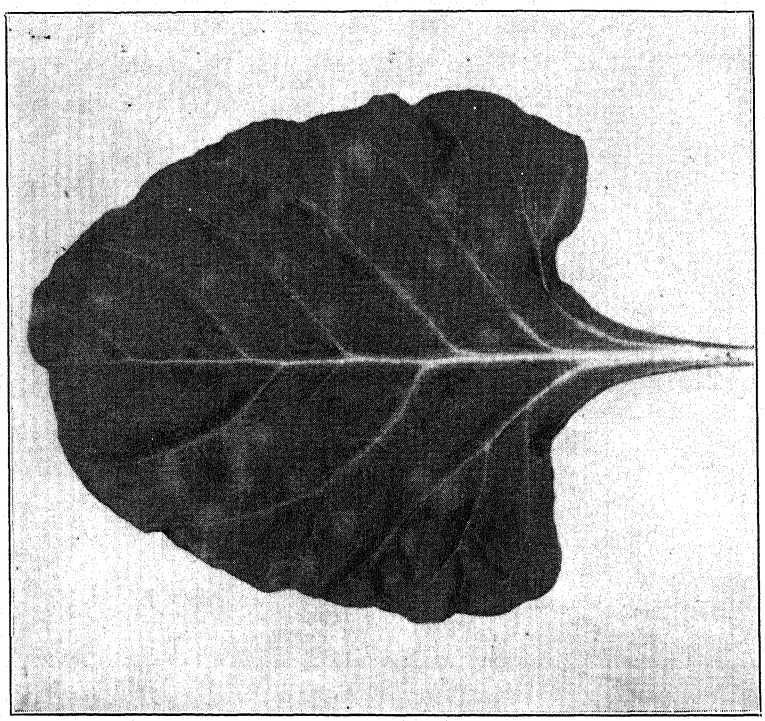


FIG. 1. Leaf of sugar beet, var. S.L. 68, with primary lesions resulting from inoculation with juice from a sugar-beet plant systemically infected by cucumber mosaic.

missible by graft but not by juice inoculation. The vector is unknown, but apparently the virus is not transmissible by the aphid, *Myzus persicae* (Sulz.).

Cucumber Mosaic. The strain of virus used was obtained from sugar beet in the vicinity of Mendota, Calif., where in at least one season it caused severe damage in a relatively large acreage of sugar beets. On leaves of sugar beet yellowish primary lesions (Fig. 1) follow juice inoculation. Systemic infection has not resulted from such lesions, but has resulted from inoculation by *Myzus persicae.*

Tomato Ringspot. The virus was obtained from field tomatoes near Riverside, California. Its relationship to other ringspot viruses of tomato

and tobacco is unknown. It produces necrosis as a primary symptom on both tobacco and tomato. Each type of plant recovers from symptoms.

Dodder Latent Mosaic. This virus was obtained from *Cuscuta californica* and was described in a recent publication (3).

Tobacco Mosaic. The virus (Tobacco virus 1 Johnson) was obtained through courtesy of Wm. N. Takahashi of the University of California, who previously had obtained it from James Johnson, University of Wisconsin.

Mustard mosaic. This virus was obtained from *Brassica adpressa* (Moench) Boiss. near Riverside, Calif., and probably is related to mild-mosaic virus of annual stock described by Tompkins (19). It is common on *Brassica adpressa* and certain other species of mustard in southern California. Systemic infection characterized by mottling occurs on *Nicotiana glutinosa* and necrotic primary lesions on inoculated leaves of Turkish tobacco.

Citrus Psorosis. H. S. Fawcett, Citrus Experiment Station, University of California, furnished this virus, which occurs on *Citrus* spp.

Peach Mosaic. L. C. Cochran, Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, supplied the virus which came from infected peach (*Prunus persica* (L.) Batsch) trees in Riverside County, California.

Tobacco Etch. The virus was obtained from tomato in a garden in Riverside, Calif. On the basis of induced symptoms and known host range it appears to be related to the tobacco-etch viruses described by Johnson (7). It produces leaflet bending, curling, and mild mottling on tomato. On Turkish tobacco the first affected leaves show yellow veins and abundant fine etching on the upper surface; leaves produced later are mottled, spotted, and stunted. Chlorotic mottling, blossom dropping, and extreme leaf distortion and narrowing are produced on jimson weed (*Datura stramonium* L.); relatively mild mottling, with some leaf puckering, is produced on tolguacha (*D. meteloides* DC.). The thermal inactivation point of the virus lies between 55° and 60° C. It is transmissible by juice inoculation and by the aphid, *Myzus persicae.*

Tomato Spotted Wilt. The virus was obtained from a tomato plant from a garden in Riverside, Calif. In the greenhouse this virus is less severe on tomato and Turkish tobacco than is usual with spotted wilt. Infected plants frequently recover.

Methods of Inoculation

Two methods were employed in making inoculations. Virus-free dodder was usually established on diseased plants and after it had grown sufficiently, stems were trained to healthy plants. Within a few hours the ends of the strands wrapped themselves around stems and petioles of the inoculated plants, and within 48 hours the dodder was sufficiently established on its new host to permit severing its connection with the diseased plant. However, as a rule, strands connecting diseased and healthy plants were maintained for longer periods, usually 7 to 10 days, to permit additional time for viruses to pass from diseased to inoculated plants.

Where it was desired to avoid contact through dodder between the diseased and inoculated plants, strands of dodder several inches long were removed from the diseased plant, placed with the basal ends in bottles filled with water, and the growing tips then were trained around succulent parts of healthy plants. Usually within 2 or 3 days such strands became established on their new host plants.

TRANSMISSION TESTS

Attempts were made to transmit each of the 12 viruses to different species and varieties of plants known to be susceptible to the virus tested. In the

TABLE 1.—*Transmission of viruses by means of Cuscuta subinclusa, C. campestris, and C. californica*

Disease induced by tested virus	Plant inoculated	Number of plants inoculated and infected by means of each species of dodder					
		Cuscuta subinclusa		*Cuscuta campestris*		*Cuscuta californica*	
		Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.
Sugar-beet curly top	Sugar beet	141	3	116	12	156	1
	Tobacco, var. Turkish	140	3	40	3	20	2
	Nicotiana glutinosa	28	0	20	0	20	1
	Tomato, var. Riverside	6	0	160	5	27	0
	Sugar beet	140	0	40	0	60	0
Sugar-beet mosaic	Sugar beet	120	0	100	0	120	0
Sugar-beet yellow vein	Sugar beet	20	0	20	0	20	0
Cucumber mosaic	Tobacco, var. Turkish	165	162	30	30	25	23
	Sugar beet	66	3	20	3	29	1
Tomato ringspot	Tobacco, var. Turkish	50	0	15	0	25	0
	Nicotiana glutinosa	20	0	15	0	15	0
Dodder latent mosaic	Sugar beet	50	50	20	20	60	60
	Pokeweed	20	20	65	65	160	160
Tobacco mosaic	Tobacco, var. Turkish	155	3	37	1	48	0
Mustard mosaic	*Brassica adpressa*	93	8	28	2	68	30
Citrus psorosis	*Citrus* sp.	10	0	10	0	.....	.....
	Tobacco, var. Turkish	30	0	.....	.....	.....	.....
Peach mosaic	Tobacco, var. Turkish	20	0	10	0	10	0
Tobacco etch	Tobacco, var. Turkish	23	0	13	0	27	27
Tomato spotted wilt	Tomato, var. Riverside	23	1	60	10	40	1
	Tobacco, var. Turkish	60	0	41	7	41	2

first experiments, *Cuscuta subinclusa* was used exclusively, but later other tests were made with *Cuscuta campestris* and *C. californica*. A partial list of the varieties and species of plants inoculated is in table 1.

The three species of dodder transmitted the virus of dodder latent mosaic to all inoculated plants of sugar beet and pokeweed (*Phytolacca americana* L.); the virus of cucumber mosaic to a high percentage of inoculated plants of Turkish tobacco; and the viruses of sugar-beet curly top and tomato spotted wilt to smaller proportions of inoculated plants. The virus of mustard mosaic was transmitted to a high percentage of plants by *Cuscuta*

californica but to a much lower percentage by the other dodder species. The virus of tobacco etch was transmitted only by *C. californica.*

Transmission of the virus of tobacco mosaic by means of *Cuscuta campestris* was reported by both Johnson (9) and Costa (5). Table 1 shows that a small proportion of plants inoculated with tobacco mosaic virus by means of *Cuscuta subinclusa* and *C. campestris* became infected. However, since accidental infection of Turkish tobacco plants by tobacco-mosaic virus is difficult to avoid in the greenhouse, and since subsequent inoculations (Table 2) in which extreme care was exercised to avoid accidental infection produced no infection, the results do not appear adequate to prove conclusively that tobacco mosaic was transmitted by dodder in these tests.

The incubation period of the viruses in inoculated plants varied considerably. With curly top the incubation period in infected sugar-beet plants was only a few days longer than that in plants inoculated by means of beet leafhoppers. Incubation periods in Turkish tobacco often were very long. In many instances no symptoms of curly top were evident until the inoculated plants began to blossom or fruit. A number of plants inoculated when 3 to 6 inches tall gave no evidence of infection even in the fruiting stage but later developed symptoms on axillary shoots after the plants were cut back to a height of about 8 inches. Incubation periods in these cases ranged from 52 to 80 days. The low percentage of Turkish tobacco plants infected by curly top by means of the 3 species of dodder (Table 1) probably is due in part to failure to hold inoculated plants for periods sufficiently long for symptoms to appear in all cases of infection.

The incubation period of dodder latent mosaic in pokeweed was relatively short, averaging about 7 days as compared to about 10 days required for production of systemic infection from juice inoculation. Also, the incubation period in sugar-beet plants inoculated by means of dodder was relatively short. Frequently the incubation period of the cucumber-mosaic virus in Turkish tobacco was no more than 6 days, which is about the time required for the production of symptoms following juice inoculation. The average incubation period of tobacco-etch virus in Turkish tobacco plants was about 8 days following dodder inoculation compared to about 4 days following juice inoculation.

Marked difference in percentages of transmission of a virus to two different host plants by means of dodder was found in only one instance. Each of the three species of dodder transmitted the cucumber-mosaic virus to high percentages of inoculated plants of Turkish tobacco but transmitted to lower percentages of plants of sugar beet (Table 1). This was true particularly of *Cuscuta subinclusa* and *C. californica.* Why dodder failed to transmit cucumber-mosaic virus to greater percentages of inoculated plants of sugar beet is not known definitely. Although this virus produced numerous primary lesions on leaves of sugar beet following juice inoculation by the rubbing method, systemic infection did not follow in any of more than 200 inoculated plants. Nevertheless, systemic infection is induced more or less

readily by the aphid, *Myzus persicae*. It is possible that to produce systemic infection it is necessary to introduce the virus into tissues of very young leaves. Aphids would be more efficient than dodder in this type of virus introduction.

COMPARISON OF ABILITY OF THREE SPECIES OF DODDER TO TRANSMIT VIRUSES

Early in the course of attempts to transmit viruses by means of dodder it became evident that species of dodder differ in their ability to transmit certain viruses. A series of tests was planned in which the three species were tested in parallel experiments using the viruses of sugar-beet curly top, cucumber mosaic, mustard mosaic, tobacco mosaic, dodder latent mosaic, sugar-beet mosaic, tobacco etch, and tomato spotted wilt. The results are in table 2.

TABLE 2.—*Comparison of ability of Cuscuta subinclusa, C. campestris, and C. californica to transmit viruses*

Disease induced by tested virus	Plant to which transfer was made	Number of plants inoculated and infected by means of each species of dodder					
		Cuscuta subinclusa		*Cuscuta campestris*		*Cuscuta californica*	
		Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.
Sugar-beet curly top	Sugar beet	80	8	80	39	80	7
Sugar-beet curly top	Tobacco, var. Turkish	80	12	80	6	80	11
Cucumber mosaic	Sugar beet	80	8	80	32	80	19
Cucumber mosaic	Tobacco, var. Turkish	60	60	60	60	60	60
Mustard mosaic	*Brassica adpressa*	100	12	100	13	100	97
Tobacco mosaic	Tobacco, var. Turkish	80	0	80	0	80	0
Dodder latent mosaic	Pokeweed	100	100	100	100	100	100
Sugar-beet mosaic	Sugar beet	80	0	80	0	80	0
Tobacco etch	Tobacco, var. Turkish	40	0	40	0	40	40
Tomato spotted wilt	*Nicotiana glutinosa*	20	0	20	14	20	1

Cuscuta campestris was the most efficient of the three species in transmission of curly-top virus to sugar beet, but there was little evidence of difference in ability to transmit this virus to Turkish tobacco. *Cuscuta campestris* was the most efficient also in transmission of cucumber-mosaic virus to sugar beet and in transmission of spotted-wilt virus to *Nicotiana glutinosa*. *Cuscuta californica*, however, transmitted the virus of mustard mosaic to a much higher percentage of plants of *Brassica adpressa* than did either of the other species. Also, it transmitted the virus of tobacco etch to all inoculated plants of Turkish tobacco; whereas, there was no transmission by either of the other species. Each species failed to transmit the viruses of tobacco mosaic and beet mosaic.

Some of these differences in the ability of the tested species of dodder to transmit viruses are extremely marked, particularly in the case of transmission of mustard mosaic, tomato spotted wilt, and tobacco etch. These results

suggest the possibility that some of the viruses not transmissible by the species of dodder used in these experiments may be transmissible by other species.

TRANSMISSION FROM PLANTS INFECTED BY TWO OR MORE VIRUSES

It is believed that dodder will prove of value in separating components of virus complexes and especially in separating one or more virus components in diseases caused by multiple infection by viruses that are not differentially transmitted by juice inoculation or by insect vectors. Already, dodder has proved useful in demonstrating double infection in a disease previously assumed to be caused by a single virus. Johnson (9) proved that the white-clover mosaic is caused by two viruses, pea-mottle virus and pea-wilt virus, in combination. The first can be separated from a mixture of the two by use of *Cuscuta campestris.*

TABLE 3.—*Transmission of viruses by means of Cuscuta subinclusa from plants infected by two or more viruses*

Virus combination	Plant inoculated	No. plants inoculated	Number of plants infected by indicated virus		
			Cucumber-mosaic virus	Tobacco-mosaic virus	Curly-top virus
Cucumber-mosaic virus plus tobacco-mosaic virus	Tobacco, var. Turkish	40	38	0	.....
Cucumber-mosaic virus plus curly-top virus	Tobacco, var. Turkish	40	40	.....	3
Cucumber-mosaic virus plus curly-top virus	Sugar beet	80	11	.....	10
Cucumber-mosaic virus plus tobacco-mosaic virus plus curly-top virus	Tobacco, var. Turkish	60	60	0	5

Since Turkish tobacco may be infected by a number of viruses in combination, tests were made using this plant as the virus source in transmission of viruses to Turkish tobacco and sugar beet by means of *Cuscuta subinclusa.* The viruses used were tobacco-mosaic virus, cucumber-mosaic virus, and curly-top virus. The combinations used and the results obtained are in table 3.

Cucumber-mosaic virus in all cases was separated from combinations with tobacco-mosaic virus and frequently from combinations with curly-top virus and from combinations of the three viruses. Of 80 small sugar-beet plants inoculated by training dodder from Turkish tobacco plants containing both cucumber-mosaic virus and curly-top virus, 11 were infected by cucumber-mosaic virus and 10 by curly-top virus. Of the infected plants, 9 were infected by cucumber-mosaic virus alone, 8 by the curly-top virus alone, and 2 by both viruses.

In other experiments, each of the three species of dodder was grown on plants infected by the viruses of dodder latent mosaic and cucumber mosaic

in combination. The dodder was then transferred at weekly intervals for 10 weeks on relatively large sugar-beet plants. All beet plants were infected by the virus of dodder latent mosaic, but only 1 plant (inoculated by means of *Cuscuta campestris*) was infected by cucumber-mosaic virus. Tests on Turkish tobacco at the end of 10 weeks showed that virus of cucumber mosaic was still present in each species of dodder. With smaller sugar-beet plants more infection by the virus of cucumber mosaic, especially by means of *Cuscuta campestris,* would be expected.

PERIOD OF ACTIVITY OF VIRUSES IN DODDER ON NONINFECTED HOST PLANTS

Several methods were used to determine the length of time viruses remained active in the three species of dodder when growing on noninfected plants.

Sugar-Beet Curly-Top Virus. Dodder was established on infected plants and trained from these to healthy sugar-beet plants known to be very resistant to infection by means of dodder because of their large size. After 3 to 5 days the strands of dodder connecting diseased and healthy plants were broken. Tests of virus content of dodder on the healthy plants were made at intervals by allowing nonviruliferous beet leafhoppers to feed on the dodder 24 hours, after which the leafhoppers were caged singly on seedling sugar beets for 7 days. Infection in the seedling beet plants indicated presence of virus in the dodder at the time of test. Virus was recovered readily from dodder 2, 3, and 5 days after the connection with curly-top plants was broken, but smaller amounts of infection after 5 days indicated decreased concentration of virus. Small amounts of virus were recovered after 7 to 10 days but no virus was recovered after longer periods. No differences were noted in the time that any one of the three species of dodder was able to retain virus.

The beet plants on which the tested dodder grew were retained in the greenhouse for more than 2 months after the tests, to determine whether any infection was produced by means of dodder. All remained free of disease. It was assumed, therefore, that these plants did not become infected and that the dodder did not pick up virus from them during the test.

Mustard-Mosaic Virus. Dodder was trained directly from diseased plants of mustard (*Brassica adpressa*) to plants of *Nicotiana glauca* Graham, believed to be immune to infection. At intervals strands of dodder were removed from the plants of *N. glauca* and established on healthy mustard plants. The first tests were made 20 days after the dodder was established on *N. glauca.* No infection of mustard was obtained by means of *Cuscuta subinclusa* or *C. campestris,* but 5 of 11 plants to which *C. californica* was transferred became infected. In tests of dodder 40 days after it was established on *Nicotiana glauca* no infection was produced on mustard by any of the three species of dodder.

Cucumber-Mosaic Virus. It has been reported (2) that *Cuscuta subinclusa* retained the virus of cucumber mosaic for long periods when growing

on an immune host. Also, Costa (5) showed that this virus remained active for 4 months in *Cuscuta campestris* growing on an immune host.

Similar results have been obtained when *Cuscuta subinclusa, C. campestris,* and *C. californica* were grown on noninfected or immune plants. Infected colonies of each of the three species of dodder were established on each of 5 large sugar-beet plants known to be very resistant to infection by the virus of cucumber mosaic owing to their size. At intervals of about 2 weeks a strand of each colony was transferred to a new plant. All dodder was removed from the older plants and these were kept for at least 2 months to determine whether they became infected. At intervals during the test and at the end of 3 months the 5 colonies of each species of dodder were tested for presence of virus. In all of these tests the dodder was found to carry virus. With 4 colonies of *Cuscuta subinclusa,* 2 colonies of *C. campestris,* and 5 colonies of *C. californica,* none of the beet plants on which the dodder grew became infected. It is assumed, therefore, that in these instances the dodder was not able to acquire additional virus from the host plants but that the virus remained active in dodder under these conditions for 3 months.

In other tests the three species of dodder contained cucumber-mosaic virus after growing 40 days on castor bean (*Ricinus communis* L.); *C. subinclusa* contained virus after growing 30 days on *Citrus* sp.; and *C. californica* contained virus after growing 40 days on *Eriogonum fasciculatum* Benth. These host plants of dodder appear to be immune to cucumber mosaic. The virus of cucumber mosaic remains active in each of the three species of dodder almost indefinitely, and probably each species of dodder is a host of the virus.

Dodder Latent-Mosaic Virus. Cuscuta californica and *C. campestris* from infected pokeweed plants were grown for 4 months on plants of *Eriogonum fasciculatum* without loss of the virus. Tests of the *Eriogonum* plants after the infected dodder was removed revealed no infection, and it is assumed that the virus was maintained for 4 months in these two species of dodder while they were growing on an immune host. It is assumed that all three species of dodder are hosts of this virus.

Tobacco-Mosaic Virus. Strands of each of the three species of dodder were removed from Turkish tobacco plants affected by tobacco mosaic, and placed on sugar-beet plants. Seven days later a strand from each of the beet plants was placed on a healthy plant of Turkish tobacco. Twenty tobacco plants were inoculated by means of each species of dodder. None became infected by mosaic. This result is not surprising in view of the small number of plants infected following direct transfer of dodder from diseased to healthy plants (Tables 1 and 2). Both Johnson (9) and Costa (5) obtained greater amounts of infection through direct transfers of strands of *Cuscuta campestris* from diseased to healthy plants. However, they failed to obtain infection by means of this species of dodder after it had been established on an immune host. It seems probable from these results that the virus of

tobacco mosaic remains active in the tested species of dodder for only a relatively short time.

Sugar-Beet-Mosaic Virus. No virus was recovered by juice inoculation from any of the three species of dodder when growing on diseased plants or after transfer to healthy plants.

Tomato-Ringspot Virus. No virus was recovered from any of the three species of dodder when growing on diseased plants or after transfer to healthy plants, either by juice inoculation to Turkish tobacco or by training dodder to Turkish tobacco and tomato.

Tobacco-Etch Virus. The three species of dodder were trained from infected plants of Turkish tobacco to plants of sugar beet, cucumber, Brussels sprouts (*Brassica oleracea* L., var. *gemmifera* DC.), and *Nicotiana glauca,* all of which appear to be immune to infection. After the dodder was established on the immune hosts it was tested for presence of virus by training strands to healthy plants of Turkish tobacco and by inoculation to Turkish tobacco by means of juice from dodder stems. No infection was obtained from *Cuscuta subinclusa* or *C. campestris* at any time by either method of test. However, inoculations by means of strands of *Cuscuta californica* trained to plants of Turkish tobacco after 2 and 4 weeks on the immune host produced infection; but no infection was produced in tests after 6 weeks. Tests with juice from the plants on which the dodder was growing produced no infection on Turkish tobacco. It is evident that *Cuscuta californica* is able to retain the virus of tobacco etch for considerable periods, although it does not appear to be a host of this virus.

RELATIVE CONCENTRATION OF VIRUS IN DODDER AND HOST PLANT

Attempts were made to compare concentrations of viruses in the three species of dodder with those in the respective infected plants on which the dodder was growing. Methods of estimating relative virus concentrations differ with the virus used, and the accuracy of the results varies with the method selected. However, in all cases, it is believed that the methods used are at least of some value in providing information on the probable relative virus concentrations. The results of these tests are in table 4 and the methods used with each virus are indicated in table 4, footnotes a to h, inclusive. All juice inoculations were made by first sprinkling the leaf with carborundum and then rubbing the surface with a cloth pad saturated with inoculum.

Beet leafhoppers obtained the virus of curly top from *Cuscuta subinclusa* in quantities that appeared equal to those obtained from the infected host plant. Smaller amounts of infection, in most cases, were produced by the leafhoppers that fed on *Cuscuta californica* and *C. campestris,* and it seems probable that the leafhoppers that fed on these species obtained somewhat less virus from these species than from the host plant. This may be due to a difference in feeding of the leafhoppers rather than to a difference in concentration of virus in host and parasite.

TABLE 4.—*Concentration of viruses in different species of dodder compared with those in diseased host plants on which the dodder was growing*

Disease induced by tested virus	Diseased plant on which dodder grew	No. infections produced by inoculations from indicated species of dodder and by inoculations from the host plant on which the dodder was growing																	
		Cuscuta subinclusa and diseased host plant						*Cuscuta campestris* and diseased host plant						*Cuscuta californica* and diseased host plant					
		Dodder in trial no.			Host in trial no.			Dodder in trial no.			Host in trial no.			Dodder in trial no.			Host in trial no.		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Sugar-beet curly top[a]	Sugar beet	20	18	18	17	19	20	17	10	12	14	17	20	19	11	8	20	17	19
Sugar-beet mosaic[b]	Sugar beet	0	0	0	48	50	50	0	0	0	50	50	50	0	0	0	50	50	50
Mustard mosaic[c]	*Brassica adpressa*	0	0	0	16	15	19	0	0	0	18	16	17	4	7	10	18	17	20
Mustard mosaic[d]	*Brassica adpressa*	0	0	0	4	11	7	0	0	0	2	8	7	0	1	3	2	14	5
Dodder latent mosaic[e]	Pokeweed	2	12	5	10	19	12	2	2	0	4	11	1	2	2	1	6	13	4
Tobacco mosaic[f]	Turkish tobacco	0.3	0	0	222	124	101	0	0	0	63	81	97	0	0	0	82	76	70
Cucumber mosaic[g]	Turkish tobacco	6	31	47	7	36	63	0	2	2	14	38	37	11	41	42	10	46	51
Tobacco etch[h]	Turkish tobacco	0	0	0	20	20	20	0	0	0	20	20	20	8	4	5	11	3	6

[a] Figures represent the number of infected sugar-beet seedlings of 20 inoculated by means of individual leafhoppers that acquired virus through a 24-hour feeding period on dodder and host plant, respectively.

[b] Figures represent number of infected sugar-beet plants of 50 inoculated by means of juice from dodder and host plant, respectively.

[c] Figures represent the number of plants of *Brassica adpressa* infected of 20 inoculated with juice of dodder and host plant, respectively.

[d] Figures represent average number of primary lesions produced on each of 3 leaves of each of 5 plants of Turkish tobacco after inoculation with juice from dodder and host plant, respectively.

[e] Figures represent average number of primary lesions produced on 3 half-leaves of each of 4 pokeweed plants inoculated with juice from dodder and host plant, respectively.

[f] Figures represent average number of primary lesions produced on each of 3 leaves of each of 3 plants of *Nicotiana glutinosa* inoculated with juice from dodder and host plant, respectively.

[g] Figures represent average number of primary lesions produced on each of 3 leaves of each of 5 plants of sugar beet inoculated with juice from dodder and host plant, respectively.

[h] Figures represent number of plants of Turkish tobacco infected of 20 inoculated with undiluted juice of *Cuscuta subinclusa* and host plant, and of *C. campestris* and host plant, respectively, and with juice, diluted 1 part juice to 1000 parts water, of *C. californica* and of host plant, respectively.

No infection by the virus of beet mosaic was obtained from juice of any of the species of dodder. It is possible, of course, that juice of dodder may tend to inactivate the virus; however, mixtures of 1 part juice of dodder (*Cuscuta subinclusa*) and 1 part juice of diseased sugar beet, undiluted, and diluted 1 to 10, and 1 to 100 with water, gave approximately the same amount of infection when inoculated into sugar beet as corresponding mixtures in which juice of healthy beet was substituted for juice of dodder.

No infection by the virus of mustard mosaic was obtained by means of juice of either *Cuscuta subinclusa* or *C. campestris*. However, by each of 2 methods of tests *Cuscuta californica* gave evidence of having appreciable concentrations of virus, although these concentrations appeared to be lower than those in the infected mustard plants.

In pokeweed and in the three species of dodder the concentration of the virus of dodder latent mosaic rises to a high level a few days after infection, then drops to a low level which is more or less constant thereafter. In tests of relative concentration of this virus, plants that had been infected several weeks were used. In these plants, in which virus content presumably had become more or less stabilized, the concentration of virus was somewhat lower in each of the three species of dodder than in the host plant.

Very few lesions were produced on inoculated leaves of *Nicotiana glutinosa* with juice from dodder growing on Turkish tobacco plants infected by tobacco mosaic. These and other tests, in which never more than 3 primary lesions were produced on a leaf of *N. glutinosa* by inoculation with juice from dodder, show that little infection can be expected from inoculations using juice of dodder from infected Turkish tobacco plants. Costa (5), however, has shown that the juice of *Cuscuta campestris* has an inhibitory effect on the virus of tobacco mosaic. Whether the juices of *C. subinclusa* and *C. californica* have a similar effect has not been determined but tests in which inoculum was prepared by adding 100 parts juice from *C. subinclusa* to 1 part juice from infected tobacco plants gave approximately the same number of lesions per leaf of *Nicotiana glutinosa* as corresponding mixtures of juice from healthy and diseased tobacco plants. It seems probable that the concentrations of virus in the three species of dodder are low as compared with those of the host plants; whether they are so low as indicated by the results presented in table 4 remains to be determined.

The concentrations of virus of cucumber mosaic in *Cuscuta subinclusa* and *C. californica* were about the same as those in the diseased tobacco plants. However, only small numbers of primary lesions were produced on leaves of sugar beet by inoculation with juice from *Cuscuta campestris*. These results are similar to those obtained by Costa (5) from inoculations with juice of *C. campestris* in which he demonstrated that juice of this species of dodder has an inhibitory action on infection. The smaller numbers of lesions produced on inoculated leaves of sugar beet, therefore, probably are due to an effect of this type rather than to a low concentration of virus. There is no evidence, however, that the juice of *C. subinclusa* or of *C. californica* produces a like inhibitory effect.

No infection was produced on Turkish tobacco by the virus of tobacco etch by juice from *Cuscuta subinclusa* or *C. campestris*. However, juice from *Cuscuta californica* produced infection on all inoculated Turkish tobacco plants. In tests in which juice of *C. californica* was diluted 1 part juice in 500, 1000 (Table 4), 2000, and 5000 parts water, respectively, and used to inoculate plants of Turkish tobacco, approximately the same numbers of plants were infected as with corresponding dilutions of juice from diseased plants of Turkish tobacco, indicating that the concentration of virus in this species of dodder was about equal to that of the infected host plant on which it was growing.

TESTS OF THE ABILITY OF DODDER TO TRANSMIT VIRUSES THROUGH SEEDS

Transmission of the virus of dodder latent mosaic through the seeds of *Cuscuta campestris* was reported earlier (3). This work was followed by

TABLE 5.—*Tests to determine whether viruses are transmitted through seeds of dodder*

Virus	Species of dodder	Plant on which tests were made	No. seeds tested	Seeds found to carry virus	
				Number	*Per cent*
Sugar-beet curly top	*Cuscuta campestris*	Sugar beet	240	0	0
Tomato ringspot	" "	Tobacco	160	0	0
Tobacco mosaic	" "	Tobacco	160	0	0
Cucumber mosaic	" "	Tobacco	480	0	0
Dodder latent mosaic	" "	Pokeweed	1340	66	4.9
Dodder latent mosaic	*C. californica*	Pokeweed	670	16	2.4
Cucumber mosaic	*C. subinclusa*	Pokeweed	240	0	0

further tests involving other viruses and two additional species of dodder.

Of the species of dodder used in these tests only *Cuscuta campestris* produced seeds in the greenhouse. This species was grown on several different types of plants each infected by a different virus and seeds were collected for test. Also, large numbers of seeds of *Cuscuta subinclusa* were collected from plants of *Nicotiana glauca* naturally infected in the field by cucumber mosaic. Seeds of *Cuscuta californica* were collected from plants on the desert shrub, *Eriogonum fasciculatum,* in an area where it was known through previous tests that a high percentage of the dodder plants was infected by the virus of dodder latent mosaic.

Seeds were planted in flats and soon after germination the seedlings were attached to young plants known to be susceptible to the virus for which tests for seed transmission were being made. The viruses used and the results obtained are in table 5. The virus of dodder latent mosaic was transmitted through 4.9 per cent of the seeds of *Cuscuta campestris* and through 2.4 per cent of the seeds of *Cuscuta californica.* Seeds of *Cuscuta campestris* still contained active virus after storage for 12 months at room temperature. None of the other viruses tested was seed transmissible.

VIRUS MOVEMENT AS INFLUENCED BY DODDER

Directional Movement in Stems of Dodder

Much evidence indicates that the movement of viruses in the phloem of plants is largely unidirectional and correlated with the movement of elaborated food materials. The evidence on which this conclusion is based was obtained from green plants in which it is not always possible to determine accurately the direction of food movement at all times. With a parasitic plant such as dodder, movement of food materials must always under normal conditions be unidirectional; that is, from the host plant into the parasite and to its regions of growth and storage. Dodder, therefore, may be better adapted for certain types of studies of the correlation between virus movement and food translocation than are green plants.

Limited studies of the movement of viruses in dodder have been made. In the first of these, virus-free dodder (*Cuscuta subinclusa*) was established on healthy plants of *Nicotiana glutinosa*. With each plant, when the first stem of dodder was about 12 inches long, it was attached to a plant of *Nicotiana glauca* infected by the virus of cucumber mosaic. When a stem of dodder from each of these latter plants was 12 inches long it in turn was attached to a healthy plant of *Nicotiana glutinosa*. The 3 plants were allowed to remain attached to each other by the strands of dodder 6 to 16 days, after which the connecting strands of dodder were broken and the plants of *N. glutinosa* were watched for development of symptoms of mosaic.

Of the 10 plants of *Nicotiana glutinosa* to which the virus-free dodder was attached at the beginning of the experiment, only 1 became infected. Hence in 9 of the 10 plants the virus did not move from the infected plant of *Nicotiana glauca* a distance of 12 inches toward the original dodder-infested plant in periods ranging from 6 to 16 days. On the other hand, all of the plants of *Nicotiana glutinosa* to which dodder became attached after making contact with diseased *Nicotiana glauca* plants became infected. Thus the virus was able to move readily in the direction of growth in the dodder stems, but moved less readily from the diseased plant along the stem of dodder to the original healthy plant on which the dodder was established.

In a second test vigorous stems of *Cuscuta subinclusa* more than 2 feet long growing on Turkish tobacco were selected. Twenty beet leafhoppers carrying the curly-top virus were caged on portions, about 7 inches long, of dodder near its points of attachment to the host plant. Twenty nonviruliferous beet leafhoppers were caged on the distal end of each stem. A section of stem, 12 inches or more in length, separated the areas of feeding of the viruliferous and nonviruliferous leafhoppers. At intervals of 1 hour for the first 6 hours and at irregular intervals thereafter, leafhoppers were removed from the cages at the distal ends of the dodder stems and caged on seedling plants of sugar beet to determine whether the leafhoppers had picked up virus.

Beginning with the third hour in some cases and with the fourth and fifth hour in others, leafhoppers removed from cages at the distal end of

dodder stems transmitted curly top to seedling plants of sugar beet. Evidently, this virus was introduced into the basal end of the dodder stem by the viruliferous leafhoppers feeding there and moved through the stem a distance of 12 inches in 3 hours in sufficient quantity to be picked up by nonviruliferous leafhoppers feeding at the other end of the stem. However, in another set of experiments where the relative positions of the viruliferous and nonviruliferous leafhoppers were reversed and leafhoppers carrying virus were allowed to feed on the distal end of the stem, the virus did not move toward the base of the stem a distance of 12 inches during 48 to 72 hours in quantities sufficient to be picked up and transmitted by the nonviruliferous leafhoppers. These results prove conclusively that the virus of curly top moves rapidly outward from the basal portion of a dodder stem in the direction of food flow, but there is no evidence of movement in the opposite direction or against the flow of food materials in periods of 2 to 3 days. This is strong additional evidence of the dependence of virus on food transport for rapid movement through the phloem.

Influence of Dodder on the Movement of Viruses from Inoculated to Noninoculated Shoots in Multiple-Crown Beets

Vigorous growth of dodder on a single infested shoot of a host plant might result in a deficit of food material in that shoot and a resultant movement of food materials from other parts of the plant into the parasitized shoot. The following experiment was made to test this theory.

Roots of sugar beet about 2 inches in diameter and about 8 inches long were split into three parts beginning at the crown and extending downward about 6 inches. They were then potted with tops equally spaced about 6 inches apart. In this manner plants, each with 3 vigorously growing tops all connected at the base of the main root, were obtained. One shoot of each plant was inoculated with a virus, dodder (*Cuscuta subinclusa*) was trained to a second shoot, and the third was retained as a control. Sugar-beet curly-top virus and sugar-beet-mosaic virus were used in the inoculations. Until symptoms of disease developed on the dodder-infested shoot, the dodder was permitted to develop unimpeded except for such pruning as was required to prevent contact with the other two beet shoots and to avoid excessive injury to the infested shoot. After symptoms of disease appeared on the dodder-infested shoot most of the dodder was removed and that which remained was kept severely pruned to avoid markedly interfering with growth of the shoot. One of the plants inoculated with curly top is shown in figure 2, and a summary of the results of these tests is in table 6.

Curly-top virus moved into shoots parasitized by dodder and produced symptoms of disease there in considerably less time than it moved into the control shoots. However, the average period for production of symptoms on the parasitized shoots varied from 32 to 46 days and was considerably longer than that required for production of symptoms on the inoculated shoots. In previous experiments (1) with the same type of beet, in which

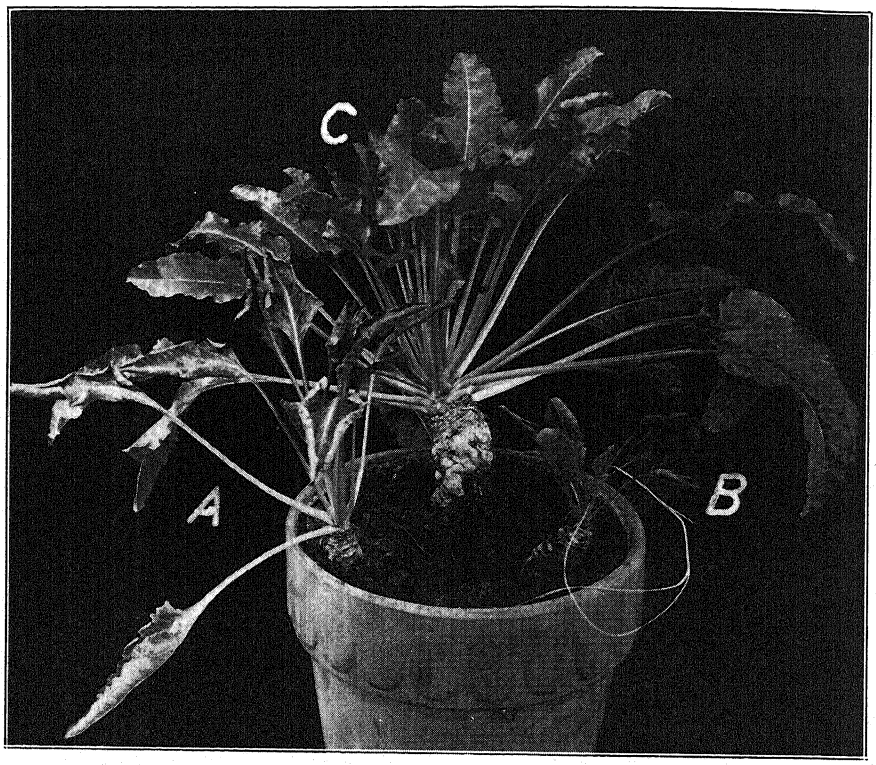


FIG. 2. Sugar-beet plant with 3 shoots on the same root system. A, Shoot inoculated with curly top by means of beet leafhoppers; B, shoot infested with *Cuscuta subinclusa* when A was inoculated; C, control. After inoculation symptoms appeared on the inoculated shoot after 13 days, on the dodder-infested shoot after 38 days, and on the control after 176 days. Plant photographed 100 days after inoculation.

TABLE 6.—*Effect of growth of dodder on movement of viruses into noninoculated shoots of sugar-beet plants split to form three separate crowns all connected through the basal part of the root*

Experiment no.	Disease induced by virus used in test	No. plants tested	Average no. days for appearance of symptoms on indicated shoot		
			Inoculated shoot	Dodder-infested shoot	Check shoot
1	Sugar-beet curly top	10	16	38	113
2	do	4	12	35	127
3	do	3	13	32	153
4	do	5	18	46	139
5	Sugar-beet mosaic	5	13	32	57
6	do	6	8	30	32
7[a]	Sugar-beet curly top	5	17	33	149
	Sugar-beet mosaic	5	12	34	41

[a] The inoculated shoot was infected with the viruses of both sugar-beet curly top and sugar-beet mosaic.

one shoot was defoliated and placed in the dark at the time another shoot was inoculated, symptoms of curly top appeared on the inoculated shoot and on the defoliated or darkened shoot at about the same time. It appears, therefore, that although the dodder greatly accelerated the rate of invasion of a parasitized shoot by the curly-top virus, growth of dodder was not so effective in this respect as removal of all foliage or exclusion of light. This probably is due to the fact that several days are required for dodder to become established sufficiently to create a food deficit in the parasitized shoot and thus induce movement of material, including virus, from the infected shoot.

The average period required for the beet-mosaic virus to move into the parasitized shoot and produce symptoms was of the same order as that required for a similar movement of curly-top virus. However, the beet-mosaic virus moved into the check shoot in a much shorter time than did the curly-top virus.

The quicker appearance of symptoms of beet mosaic on the check shoot probably is due to the types of tissue in which movement takes place. Apparently movement of either virus into the check shoot through the phloem can be prevented so long as a continuous flow of food materials out of this shoot is maintained. Since the virus of curly top apparently moves readily only in the phloem, it may be prevented in this way from entering the check shoot for an indefinite period. However, the virus of beet mosaic, in addition to being able to move through the phloem in the direction of food transport, also is able to move slowly, but at a more or less uniform rate, through the parenchyma, probably by diffusion aided by protoplasmic streaming. Average periods of 32 to 57 days required for the production of symptoms of mosaic on the check shoots probably are long enough to enable the virus of beet mosaic to pass into the check shoots by movement through the parenchyma alone. As in the case of the curly-top virus, however, the virus of beet mosaic can be induced to move rapidly through the phloem into noninoculated shoots by defoliating the shoots or by placing them in the dark.

MECHANICS OF TRANSMISSION OF VIRUSES BY DODDER

Some viruses, such as that of dodder latent mosaic, were transmitted to all inoculated plants by each of the three species of dodder; whereas, others, such as the virus of beet mosaic, were not transmitted. With certain viruses there is considerable variation among the species of dodder in ability to transmit. For example, the virus of tobacco etch was transmitted to all inoculated plants by *Cuscuta californica* but not by *C. subinclusa* or *C. campestris*. All of the factors responsible for these variations are not clear but it seems probable that the differences may be explained in part on the basis of the anatomical relationships of dodder to the host plant coupled with the relationship of different types of viruses to the tissues of the host plant and to dodder.

Anatomy of the Haustorium and Its Relationship to the Host

Since viruses that are acquired by or are introduced into a plant through the medium of dodder must pass through the haustorium, the anatomy of the haustorium and its relationship to the host plant are of extreme importance in any attempt to visualize the mechanics of acquisition or transmission of viruses by dodder.

The formation of the haustorium of *Cuscuta* was described by Peirce (15) and by Thoday (18). According to these investigators, sucker-like processes arise from the epidermis of the dodder stem and adhere firmly to the host plant. A true haustorium, regarded morphologically as an adventitious root, then begins to develop in the cortex in the region of the pericycle. When this comes in contact with the host numerous surface cells of the haustorium develop into elongated hypha-like strands that penetrate deep into the tissue of the host. In this process host cells are absorbed and the strands of the central portion of the haustorium fuse into a more or less compact tissue. Some of the central strands make contact with the xylem and become differentiated into tracheids; others make contact with the phloem.

All investigators agree there is an unbroken tracheal connection between host and parasite through the mature haustorium. This is easily demonstrated even in free-hand sections in *Cuscuta subinclusa* in sugar beet and tobacco. The character of the connection with the phloem, however, is not so clearly evident. Thoday (18) states that in the species of *Cuscuta* studied by her the hypha-like cells of the parasite enlarged at the tip as they approached sieve tubes of the host and the cell walls became plastic and gelatinous. The cell walls at the tips seemed to disappear and the protoplasm applied itself directly to the wall of the sieve tube of the host usually either over a lateral sieve plate or a sieve field. The strands then developed into sieve tubes with short cells.

In *Cuscuta odorata* on *Pelargonium zonale* (L.) Willd., Schumacher (16) found that the ends of elongated feeding cells made contact with the host sieve tubes and flattened out and elongated in one or both directions parallel to the long axis of the sieve tube. Numerous finger-like processes grew out and firmly clasped the sieve tube. Thin walls of the cells of the parasite were directly attached to the unbroken wall of the sieve tube and no evidence of lateral sieve plates or of plasmodesmata connecting host with parasite was obtained. The feeding strands were not observed to differentiate into sieve tubes in the haustorium. In passage from the sieve tubes of the host to those of the parasite it would be necessary under these conditions for materials to move through the cell wall of the host sieve tube, the cell wall of the feeding cell of the parasite and through parenchyma-like tissue traversing the entire length of the haustorium.

Sections of petioles of sugar beet and stems of *Nicotiana glauca* and Turkish tobacco, parasitized by *Cuscuta subinclusa,* were prepared and studied by Katherine Esau of the University of California. She[3] found

[3] Letter to the writer.

cells in the haustorium that had no nuclei but possessed sieve plates with evident callus cylinders. Like typical sieve-tube elements, these cells contained little chromatic material and had companion cells. These sieve tubes were traced to their union with the long hypha-like cells that in their turn connected with the phloem of the host and formed among the phloem cells the finger-like processes described by Schumacher (16). The method of union between the haustorial cells and the sieve tubes of the host was not ascertained.

Regardless of the technical terminology that should be used to designate the food-transporting tissue throughout the length of the haustorium, it is evident that within the haustorium there is a tissue that functions in a highly efficient manner in the transport of materials from the host to the parasite. *Cuscuta subinclusa* on tobacco sometimes produces a stem elongation of 3 inches or more in 24 hours. This amount of growth would involve the transport of relatively large volumes of material. Schumacher (16), on the basis of growth rates and cross-sectional areas of the haustorium, calculated that materials moved out of the host plant into *Cuscuta odorata* at a rate of from 1.6 to 6.2 mm. per hour where movement was considered to take place through the total cross-sectional area of the haustorium. However, since movement probably takes place through only a limited part of the total cross-sectional area of the haustorium, actual rates may be far in excess of those indicated by these figures.

The haustorium of *Cuscuta subinclusa* makes a close union with the tissues of sugar beet and tobacco. The line of demarcation between host and parasite is evident toward the surface of the host tissue but in the vascular region and in the part immediately outside of the vascular region union is so close that it is difficult, in some cases, to differentiate between cells of the parasite and those of the host.

In *Cuscuta odorata* Schumacher and Halbsguth (17) found that the long, hypha-like, terminal cells that radiate from the haustorium and grow through the parenchyma of the host have what they interpreted as numberless fine plasma threads in their walls that pass through the walls to their outer surfaces. Such threads were lacking, however, in walls of cells that attached themselves directly to the sieve tubes of the host.

In sections of the haustorium of *Cuscuta subinclusa* in sugar beet and tobacco with which a staining technique to show plasmodesmata was employed, Esau[4] found darkly stained granules in linear series traversing the walls of haustorial cells that penetrated into the cortical parenchyma of the host. Students of plasmodesmata commonly interpret such lines as a result of the precipitation of the stain (gentian violet in this instance) in places where cytoplasm was present in the living state. If this interpretation is correct, the granular lines within the walls of the host and parasite cells indicate the position of plasmodesmata in these walls. The arrangement and density of these lines are illustrated in figure 3. They are numerous and

[4] Letter to the writer.

rather uniformly distributed in the haustorial cell walls and are continuous from the lumen of the haustorial cell to the lumen of the invaded host cell. In the parenchyma cells of the host the lines are numerous in some walls, few or absent in others, and often are confined to the pit areas. In figure

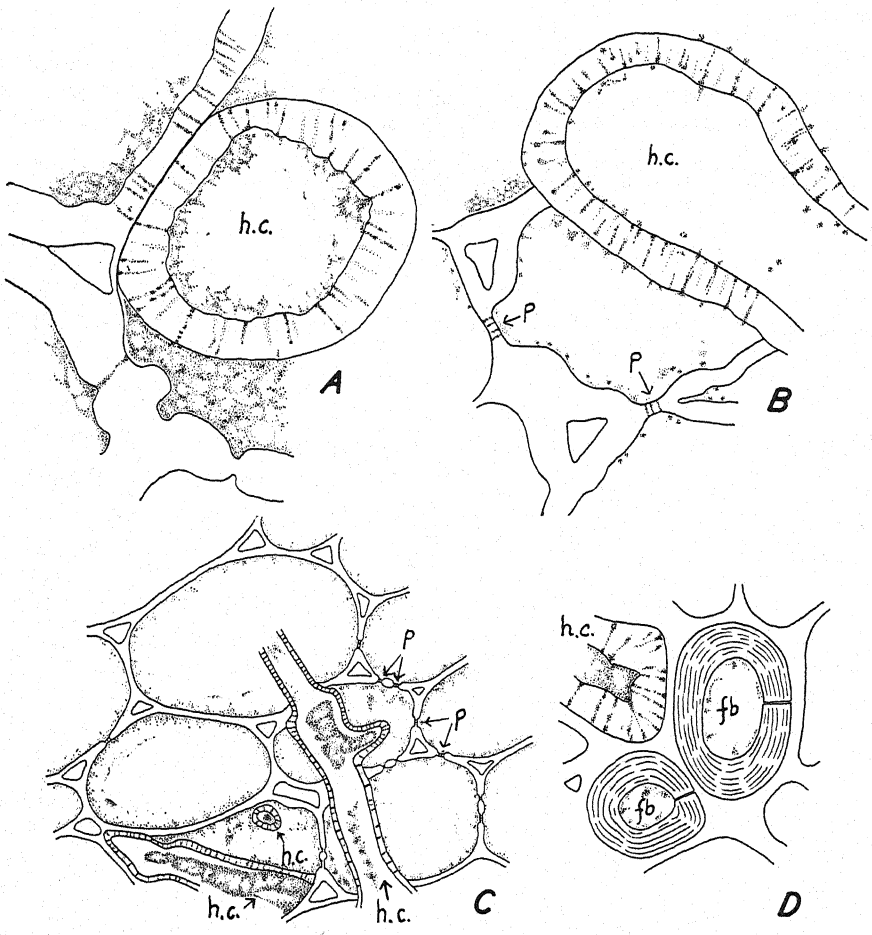


FIG. 3. Free-hand sections of tobacco and sugar-beet stems and petioles showing the relation of the haustorial cells of *Cuscuta subinclusa* to the cells of the host. Plasmodesmata are in the haustorial cells and in the walls of the host plants, either scattered throughout the wall (A) or concentrated in the pit areas (B and C at p). A, Haustorial cell in transverse section adjacent to a wall of a parenchyma cell of *Nicotiana tabacum;* B, end of a haustorial cell and a portion of a parenchyma cell of *Nicotiana glauca;* C, sugar-beet parenchyma with sections of three haustorial cells; D, branch of haustorial cell adpressed to the walls of a primary-phloem fiber (fb) of *Nicotiana glauca,* secondary walls of the fibers marked by concentric lines. fb, fiber; h.c., haustorial cell; p., pit. A, B, and D × 730; C × 163. Sections and drawings by Katherine Esau.

3, A, lines passing through the walls of the outermost cell of the haustorium meet similar lines traversing the wall of the adjacent host cell.

All haustoria illustrated in figure 3 occurred within the parenchyma tissues of the host. In the walls of the ultimate ramifications of the haus-

torial cells within the phloem of the hosts no plasmodesmata were identified with certainty.

If these darkly-stained lines traversing the walls of the peripheral cells of the haustorium of *Cuscuta subinclusa* and similar structures in the walls of hypha-like strands of *Cuscuta odorata* represent plasmodesmata, it is evident that the protoplasm of the parasite may provide direct contact with protoplasm of the host through the medium of these protoplasmic strands passing through the cell walls. This is of special significance for the transmission of certain types of viruses by dodder, since there is strong evidence pointing to plasmodesmata as the avenues of virus movement in the passage of viruses from cell to cell in the parenchyma of flowering plants.

Movement of Viruses into Dodder

If food movement is the result of flow of liquid content of the phloem from points of higher pressure to those of lower tension as suggested by Münch (14) and by Crafts (6), any material, including virus, not filtered out by passage of the liquid through intervening cell walls or membranes, would pass readily from the phloem of diseased plants into dodder. The concentration of virus in the phloem of dodder soon would equal or surpass that in the phloem of the host if not rapidly inactivated in the phloem of dodder. The concentration of virus in dodder, therefore, probably would be influenced decidedly by the concentration of virus in the phloem of the host plant.

It may be assumed with considerable certainty that the virus of curly top is closely limited to the phloem of its host plants. This may be true also for most of the "yellows" type of viruses that produce disturbances in the phloem but which do not produce mottling, rings, local lesions, or other types of disturbances that indicate a direct effect on the parenchyma. Viruses causing mottling, various types of lesions, and other types of disturbances in parenchyma, on the other hand, occur in both phloem and parenchyma, although there is some evidence that the concentration of such viruses in the phloem, in some cases at least, may be low compared with that in the parenchyma.

On the basis of this reasoning it would be expected that phloem-limited viruses, as a rule, would pass into dodder in relatively high concentrations. Also it would be expected that mosaic viruses would move into dodder in lower concentrations and, if unable to invade parenchyma of dodder and accumulate concentrations of virus there, often would be found in low concentrations in dodder, especially if the rate of inactivation in dodder were relatively high. This may account for the very low concentration or absence of the virus of tobacco mosaic, beet mosaic, and tomato ringspot in dodder growing on diseased plants. In cases, however, where dodder is a host of the invading virus, such viruses would multiply in the dodder and reach a high concentration. In certain cases where dodder is not a host of the virus it may be that appreciable concentrations of virus are reached in dodder by invasion of parenchyma and temporary storage in this type of tissue.

It is possible also that mosaic viruses may enter dodder through the parenchyma contacts of the haustorium with the host; but, since this type of entry involves movement through a considerable amount of parenchyma tissue in which movement is slow, it would not be expected to influence appreciably the concentration of virus found in the dodder.

Movement of Viruses Out of Dodder

There appear to be three ways by which it is possible for viruses to move from dodder into healthy host plants and produce infection: (a) The virus may move through the phloem or food transport cells of the haustorium counter to the prevailing direction of food transport and become established in the host plant where it would be able to multiply and produce systemic infection. (b) There is some question as to whether or not the tips of some of the hypha-like strands of the haustorium that penetrate the host cells have well-defined cell walls. If the growing tips of these structures are naked protoplasm and susceptible to infection, virus may pass by way of these directly from the protoplasm of the parasite into the protoplasm of the host and continue to move through the parenchyma tissue of the host until a systemic infection is produced. (c) If the structures observed traversing the walls of haustorial cells of *Cuscuta subinclusa* and *C. odorata* are plasmodesmata it would seem possible for any virus able to invade the outer parenchyma cells of the haustorium to move along these protoplasmic strands and enter the host protoplasm either through similar strands penetrating the walls of adjacent host cells or by passage into host protoplasm applied to the outer surface of walls of the invading hypha-like cells. Invasion of the host parenchyma and eventually of the entire plant could then take place.

The path taken by a virus in passing from dodder to a host plant, therefore, would be determined largely by its relationship to the different tissues of dodder and the host plant. It would be expected that the phloem-restricted (yellows type) viruses and parenchyma-inhabiting (mosaic type) viruses would have rather marked differences in relation to their transmissibility by dodder.

Viruses of the Yellows Type. The path available for passage of phloem-limited viruses would seem to be largely or wholly restricted to specialized transport cells (sieve tubes?) of the haustorium. At what stage in haustorial development this pathway would become available for virus movement into the host is problematical. If the hypha-like cells of the haustorium that later develop into food transport cells are subject to invasion by virus, it is possible that infection of the host may occur at the time contact between these hypha-like cells and the sieve tubes of the host is made, or soon after. If virus is unable to pass into these young haustorial cells before they develop into transport cells, as seems more likely, and infection is delayed until movement of food materials out of the host begins, infection then would involve movement of virus out of dodder into the host plant counter to the

general direction of movement of elaborated food materials. However, this movement would be through a very short distance, for when dodder is trained from a diseased to a healthy plant the virus continues to pass, for a time at least, from the diseased plant along the stem of dodder past the new haustoria developing in the healthy plant. A virus such as that of curly top, moving from the diseased plant, would pass through the phloem in the strands of dodder within a millimeter, more or less, of the phloem of the healthy plant. Moreover, so long as the virus retained its activity it would be present in the phloem of dodder only a short distance from the phloem of the new host. The fact that virus of curly top did not bridge this short distance and produce infection in dodder-inoculated plants more often than was found is evidence of a high degree of efficiency on the part of dodder in maintaining a condition of continuous flow of material out of the host plant.

The frequency with which such viruses are able to pass through the haustorium and enter the host plant probably depends on concentration of virus in the dodder, duration of period of activity of virus in the phloem of dodder, and perhaps on other factors such as temporary slight reversals of food flow between dodder and host. Since phloem-limited viruses probably occur in relatively high concentrations in the phloem of their hosts, it would be expected that they would enter dodder in relatively high concentrations. All such viruses that retain their activity for appreciable periods of time in the phloem of dodder should be transmissible; but on the basis of the evidence regarding the difficulty of movement of virus through the haustorium counter to the direction of food flow they should be transmissible, as a rule, to relatively low percentages of the inoculated plants except in cases where dodder is a host of the virus, in which case high percentages of infection should be obtained, but perhaps only after longer periods of contact between the infected dodder and healthy host plant.

These conclusions are supported by the available results of tests with *Cuscuta campestris* (Table 7). Of the 19 viruses listed, 7 are classified as belonging to the yellows group and all of these are transmissible by *Cuscuta campestris*. Four of the 6 viruses on which data are available, were transmitted to 26 per cent of inoculated plants or less. Dodder has not been shown to be a host of any of these 4 viruses. A high percentage of infection was obtained with each of the 2 remaining viruses. Of these latter, Costa (5) has shown that the virus of cranberry false blossom multiplies in *Cuscuta campestris;* and the high percentage of plants infected by potato witches'-broom virus suggests that *C. campestris* also may either be a host of this virus or able to retain active virus for a long time. On the basis of these results it may be expected that many other viruses of the yellows type will prove to be transmissible by dodder.

Viruses of the Mosaic Type. If present in comparable concentrations in the phloem of dodder, it would be expected that mosaic viruses would be able to produce infection as readily as yellows viruses by moving through food transport cells of the haustorium into the host counter to the direction of

movement of materials into the parasite. However, since there is evidence that some of the mosaic viruses occur in low concentrations in the phloem of their host plants, it may be that as a rule these viruses are picked up in low concentrations by dodder. Furthermore, if the phloem of normal host plants is an unfavorable medium for such viruses, the phloem of dodder should prove to be an even more unfavorable medium, and after passage into dodder the initial low concentration of virus probably would be reduced still further by inactivation. Thus mosaic viruses not only may enter the phloem

TABLE 7.—*Summary of information available on transmission of viruses by Cuscuta campestris*

Disease induced by tested virus	Plant inoculated	No. plants inoculated	Plants infected		Authority[a]
			Number	*Per cent*	
Yellows type:					
Sugar-beet curly top	Sugar beet	196	51	26	.....
Sugar-beet yellow wilt	Sugar beet	17	4	24	(4)
Aster yellows	Aster	50	4	8	(9)
Tomato bushy stunt	Tomato	20	5	25	(9)
Cranberry false blossom	Tomato	93	69	74	(5)
Peach rosette	Tomato	Not stated	+[b]	?	(12)
Potato witches'-broom	*Vinca rosea*	48	42	82	(13)
Mosaic type:					
Sugar-beet mosaic	Sugar beet	180	0	0	.....
Cucumber mosaic	Tobacco, var. Turkish	90	90	100	.....
Mustard mosaic	*Brassica adpressa*	128	15	12	.....
Tobacco mosaic	Tobacco, var. Turkish	117	1[c]	1	.....
Tomato ringspot	Tobacco, var. Turkish	40	0	0	.....
Tobacco etch	Tobacco, var. Turkish	40	0	0	.....
Dodder latent mosaic	Pokeweed	120	120	100	.....
Tomato spotted wilt	Tobacco, var. Turkish	41	7	17[d]	.....
Citrus psorosis	Citrus sp. seedlings	10	0	0	.....
Tobacco ringspot	Tobacco, var. Turkish	32	0	0	(9)
Pea wilt	*Medicago lupulina* L.	8	5	62	(9)
Pea mottle	*Medicago lupulina*	8	0	0	(9)

[a] Where authority is not indicated, results are those reported in this publication in tables 1 and 2.

[b] Plus sign indicates that infection was obtained but number of infected plants was not stated.

[c] However, Johnson (9) reported infection in 13 of 26 Turkish tobacco plants and Costa (5) reported infection in 5 of 31 tomato plants inoculated by means of *Cuscuta campestris*.

[d] In limited tests a higher percentage of infection was obtained on *Nicotiana glutinosa* (Table 2).

of dodder in low concentrations but may tend also to be lost rapidly from the phloem, possibly in some cases before they pass through considerable lengths of stems of dodder. For these reasons it may be that infection by mosaic viruses by passage from dodder to the host plant through the food transport cells of the haustorium would occur less often than with yellows viruses that may occur in higher concentrations in the phloem and be better adapted to survive in this type of tissue.

However, mosaic viruses invade the parenchyma of their host plants and reach relatively high concentrations in such tissue, and it is probable that

transmission of mosaic viruses to high percentages of inoculated plants will be found associated with ability of these viruses to invade parenchyma of dodder. Invasion of parenchyma would enable virus to move through the haustorial tissues to the peripheral cells of the haustorium from which it would be able to move through the plasmodesmata traversing the walls of cells in or adjacent to those of the host and invade the protoplasm of the host plant. As already suggested, it is possible also that if the tips of young invading hypha-like cells of the haustorium are devoid of cell walls and contain virus, infection by mosaic viruses may take place by direct passage of virus from the invading protoplasm to that of the host.

In view of the evidence indicating low concentration of mosaic viruses in the phloem of dodder, occurrence of such a virus in relatively high concentration in the juice of dodder should be evidence of its ability to invade parenchyma of dodder. Therefore, all mosaic viruses that occur in relatively high concentrations in expressed juice of dodder should be transmissible by dodder to high percentages of inoculated plants.

These conclusions are supported by information on transmission of parenchyma-inhabiting viruses by means of *Cuscuta campestris* presented in table 7. Of the 12 viruses listed, 2 were transmitted to 100 per cent of inoculated plants, 1 to 62 per cent of inoculated plants, 3 to small percentages of inoculated plants, and 6 to none of the inoculated plants. *Cuscuta campestris* appears to be a host of both of the viruses transmitted to 100 per cent of inoculated plants. No information is available regarding the relationship to *C. campestris* of the virus transmitted to 62 per cent of inoculated plants. There is no evidence that the viruses of any of the other diseases transmitted to low percentages of inoculated plants or nontransmissible occur in dodder in any appreciable concentration. Also, with *Cuscuta subinclusa* and *C. californica* there is a marked correlation between indicated concentration of virus in dodder (Table 4) and percentage infection produced by means of dodder on inoculated plants (Tables 1 and 2). Strong additional support for these concepts is afforded by the fact that *Cuscuta californica* had relatively high concentrations of virus of tobacco etch, transmitted the virus from diseased to all inoculated plants of Turkish tobacco, and retained virus 4 weeks while growing on immune hosts; whereas, in parallel tests no virus was recovered from either *Cuscuta subinclusa* or *C. campestris* and no infection was obtained by means of either of these species.

With the mosaic type of virus, therefore, it may be expected that as a rule transmission by means of dodder will prove to be either very high or very low or absent. Those viruses that are able to utilize dodder as a host or that are able to invade the parenchyma of dodder and retain their activity for considerable periods in such tissue will be transmissible to a high percentage of inoculated plants; whereas, those not able to utilize dodder as a host or to invade the parenchyma of dodder will be, as a rule, either not transmissible or transmitted sporadically.

SUMMARY

Transmission of 12 plant viruses by three species of dodder, *Cuscuta subinclusa, C. campestris,* and *C. californica,* was studied. The viruses of dodder latent mosaic and cucumber mosaic were transmitted to high percentages of inoculated plants by each of the three species of dodder. The viruses of curly top and spotted wilt were transmitted to smaller proportions of inoculated plants. The virus of mustard mosaic was transmitted to high percentages of inoculated plants by *Cuscuta californica* but to low percentages by the other two dodder species. The virus of tobacco etch was transmitted to all inoculated Turkish-tobacco plants but was not transmitted by either of the other species. No transmission of the virus of tobacco mosaic was obtained with *Cuscuta californica* but a very low percentage of Turkish tobacco plants inoculated by means of the other two species became infected. No transmission was obtained of the viruses of sugar-beet mosaic, sugar-beet yellow vein, tomato ringspot, citrus psorosis, or peach mosaic by any of the species of dodder.

In tests to determine longevity of viruses in dodder growing on noninfected host plants, the viruses of cucumber mosaic and dodder latent mosaic were active in the three species of dodder after periods of 1 to 4 months. The virus of curly top was not recovered in periods longer than 10 days. The virus of mustard mosaic was active in *Cuscuta californica* after 20 days but not after 40 days; apparently it was lost from *C. subinclusa* and *C. campestris* in shorter periods. The virus of tobacco etch persisted in *Cuscuta californica* for periods of 2 to 4 weeks but was not recovered from the other two species.

Tests of relative concentration of virus in dodder and infected plants on which the dodder was growing indicated that the concentrations of curly top virus in dodder were about equal to or slightly less than those in diseased sugar beet. Concentrations of the virus of dodder latent mosaic were somewhat lower than in diseased pokeweed plants. Concentrations of cucumber-mosaic virus were about equal to those of the host in *Cuscuta subinclusa* and *C. californica,* but little virus was recovered from juice of *C. campestris,* possibly because of inhibitory effects of juice of this species. Concentrations of the virus of mustard mosaic in *Cuscuta californica* were considerably lower than those of the host; no virus was recovered from *C. campestris* or *C. subinclusa.* Concentrations of virus of tobacco etch appeared to be as high in *Cuscuta californica* as in the host but no virus was recovered from the other two species of dodder. Concentration of the virus of tobacco mosaic appeared to be low in each species of dodder as compared with the host. No virus was recovered from dodder growing on plants infected by sugar-beet mosaic, sugar-beet yellow vein, or tomato ringspot.

The virus of dodder latent mosaic was transmitted through 2.4 per cent of the seeds of *Cuscuta californica* and through 4.9 per cent of the seeds of *C. campestris.* The virus was active in seeds of the latter species after a storage period of 1 year.

The movement of the viruses of curly top and cucumber mosaic in dodder stems was much more rapid toward growing points and away from the host than in the opposite direction. Growth of dodder on one shoot of beet plants with three shoots on a single root system induced movement of the curly-top virus from an inoculated shoot in periods of from 32 to 46 days; whereas, check shoots remained free of symptoms for periods of 113 to 149 days.

The tracheal elements of dodder make direct contact with those of the host through the haustorium. There is disagreement regarding the nature of the cells uniting the phloem of the parasite with that of the host but it is evident that food materials move rapidly through these cells and that they function as phloem. The walls of certain outer cells of the haustorium are traversed by lines that appear to be plasmodesmata. These in some cases appear to join with similar lines in walls of adjacent host cells.

Viruses appear to be acquired by dodder mainly by movement from the host into the parasite through the phloem with the food materials. Infection probably takes place: (a) by movement of virus from the phloem of dodder through the haustorium into the phloem of the host counter to the prevailing direction of food movement, and (b) by passage from the parenchyma of the haustorium into that of the host through plasmodesmatal strands or from naked protoplasm of invading hypha-like cells. It is suggested that infection by yellows-type viruses may be effected by the first method and that infection by mosaic-type viruses may be effected chiefly by the second method. An analysis of the available information on transmission of viruses by *Cuscuta campestris* shows that all tested viruses classified as yellows types are transmitted but, in general, to low percentages of plants; whereas, relatively few of the mosaic-type viruses are transmissible but those that are transmitted in general are transmitted either to a very high or to a very low percentage of inoculated plants.

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REPORT OF THE TWENTY-SEVENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 27th annual meeting of the Pacific Division of the American Phytopathological Society was held at Oregon State College, Corvallis, June 26 to 28, 1944. Thirty-two members and 12 non-members from Washington, Oregon, California, Idaho, Nevada, and Washington, D. C., attended the scientific sessions and a lesser number attended the two field trips. Twenty-two papers were presented. At the business meeting it was voted to purchase three $100 War Bonds in the name of the American Phytopathological Society from the funds of the Pacific Division.

Officers for 1945 were elected as follows: President: B. L. Richards; Vice-President: H. R. McLarty; Secretary-Treasurer: Geo. W. Fischer; Councilor: L. D. Leach; Representative, Pacific Division A.A.A.S.: L. D. Leach.

Abstracts of papers presented follow.

C. E. YARWOOD,
Secretary-Treasurer.

Further Evidence of Pollen Dissemination of Walnut Blight. P. A. ARK. In the spring of 1944, many recently pollinated nuts (3 to 5 mm. in size) in a commercial walnut orchard were completely blighted by *Phytomonas juglandis.* No foliar blight and hold-over lesions occurred on the majority of trees examined. This condition prevailed on both Bordeaux-sprayed and unsprayed trees. Wash water from apparently healthy leaves and nuts from trees in the blighted orchard yielded virulent cultures of *P. juglandis.* Young Northern California black walnut trees, although planted 25 to 50 feet from blighted Payne trees had numerous leaf infections from the tree top to the ground line. However, no cankers or blighted dormant buds could be detected on these trees. In the absence of hold-over lesions, it is assumed the disease was induced by walnut pollen grains abundantly present on the leaves. Since many nuts are initially blighted at the apical end, and because some well-separated, young, non-bearing trees develop foliar blight in the absence of overwintering foci of infection, it is believed the high incidence of the disease this spring may be attributed to contaminated wind-borne walnut pollen, originating in partially blighted catkins of the highly susceptible Payne variety.

Bacterial Blight of Purple Vetch Caused by Phytomonas pisi. P. A. ARK. In a field of several acres in Sonoma County, California, purple vetch (*Vicia atropurpurea* Desf.) and Canadian field peas (*Pisum sativum* L. var. *arvense*) were found severely blighted by *Phytomonas pisi.* In many cases the disease on the vetch originated at the middle of the stem, spread rapidly toward the base, and quickly killed the plant. Occasionally only the tips of the plants were blighted, and the progress of the disease probably was checked by an increase in temperature. Pea plants developed blight lesions with isolates from both hosts, and successful cross-inoculation was obtained in the greenhouse.

Phytophthora Root Rot of Guayule. ALVIN J. BRAUN. A root rot caused by *Phytophthora drechsleri* Tucker has been observed in many irrigated field plantings of guayule in California, especially on the heavy, poorly drained soils. The greatest loss occurs on the clay soils of the Tracy-Newman district where root rot was observed in 22 of the 25 fields surveyed in September, 1943. Losses of 5 per cent or more of the plants were recorded for 625 of the 3745 acres included in the survey. When the lesion girdles the tap root the plant wilts rapidly, the leaves turn gray, dry, and usually remain firmly attached to the plant for the remainder of the season. The root lesions are black, sunken, and firm, the upper margin usually being 2 to 6 inches below the ground line. Field observations and temperature tank studies show that soil temperatures above 60° F. are necessary for infection. Soil moisture in excess of the field capacity for extended periods is particularly conducive to the development of root rot. This condition is more readily attained on the heavy, poorly drained soils and on soils underlain with a claypan or hardpan. Control is effected by avoiding soil moisture conditions favorable to infection at times when the soil temperature at the 6-inch depth is above 65° F.

Black Streak, a Bacterial Disease of Sugar Beet in the Pacific Northwest. EUBANKS CARSNER. A bacterial disease characterized by black or dark brown lesions on leaf blades, petioles, and seed stalks of sugar beet has been observed near Gazelle, Ft. Jones, and Montague in northern California, in the vicinity of Mt. Vernon in western Washington, and for several years and in many places in the Willamette and Rogue River valleys of Oregon. The causal organism apparently enters through hydathodes and through wounds.

Frequently it invades tissue injured by downy mildew. Field observations suggest that the causal organism may be seed borne. Sugar beet varieties vary in susceptibility to black streak, but in no field observed has the injury been a major factor affecting seed yield. The causal bacterium is probably the same as that described as *Bacterium aptatum* by Nellie A. Brown and Clara O. Jamieson (Jour. Agr. Res. 1: 189–210. 1913).

The "Complex Concept" of the Peach Mosaic and Certain Other Stone Fruit Viruses. L. C. COCHRAN. Several years' study has shown that peach mosaic virus from individual sources produces uniform symptoms on Hale peach, but the same virus from separate sources varies extensively in intensity and type of symptoms. Viruses from different sources were previously arbitrarily divided according to effect into severe, medium, and mild categories and were called strains. Further studies indicate the existence of infinite categories and, given enough sources, these overlap to form a symptom gradient in which no definable strains can be segregated. When peach mosaic virus from various sources is placed in apricot and certain other stone fruits, a severe to symptomless gradient results which may or may not agree with the severity in peach. Mild to severe symptom gradients are also known for ring spot, asteroid spot, and certain of the cherry viruses. If certain viruses are groups there is need for group terminology. Following a suggestion of C. W. Bennett, it is proposed that the term *strain* be reserved for variants that are sufficiently definable to be recovered from nature and identified. It is further proposed that the term *form* or *isolate* with appropriate descriptive adjectives be used for viruses from individual sources which vary widely in symptom expression. Collectively such viruses may be referred to as *complexes*.

Some Host-tissue Relationships of the Peach Mosaic Virus. L. C. COCHRAN and JOHN L. RUE. Evidence that the peach mosaic virus invades all living parts of the peach tree except seeds has been obtained through transmission with infected leaf, fruit, bark, and wood tissue. Inoculations were made by placing the infected tissue under the bark in T cuts, as in budding. Some leaf sections were green 75 days after inoculation and appeared to have united with the wound callus. Some of the fruit sections remained firm and green 25 days after inoculation. Transmission was accomplished with green, hard ripe, and with firm juicy ripe peach fruit tissue but not with soft ripe, or dried fruit, or with pieces of integuments or embryo. Fruit tissue from infected plums, apricots, and almonds and wood shields cut from apricot and peach twigs, from which the cambium had been removed, transmitted to peach. The incubation period of the peach mosaic virus in peach varies proportionately with the growth condition at the time of inoculation. Trees inoculated when breaking dormancy and those with 1-inch leaves developed symptoms in 14 and 50 days, respectively. Trees with initial leaves fully expanded usually developed symptoms only in shoots in line with and below the inoculation point. Trees inoculated after June usually developed no symptoms until growth started the following year.

The Mode of Infection and the Incubation Period in the Stem Smut of Grasses, Ustilago hypodytes. GEORGE W. FISCHER. During intensive observation and experimentation on stem smut at Pullman, Washington, it has been discovered that infection occurs in vegetative parts of the host and that the incubation period is at least 2 years and more often 3 years. In planted stands of crested wheatgrass (*Agropyron cristatum*) and other grasses, in no case has stem smut appeared before the third heading of the grass. All blossom and seed inoculations with this smut have given purely negative results. Infection was obtained in crested wheatgrass, slender wheatgrass (*A. trachycaulum*), and Canada wild rye (*Elymus canadensis*), with stem smut from several species of *Agropyron* and *Elymus* only by inoculation of mature plants. These had been clipped back after heading in 1939, sprayed with suspensions of smut spores, and then covered with wet burlap for 48 hours. In 1941, a slight amount of stem smut was noted in some of the slender wheatgrass and crested wheatgrass. In 1942 stem smut was abundant in nearly all rows of slender wheatgrass and crested wheatgrass, and in all of the Canada wild rye. The source of infection of the many species of *Agropyron, Elymus, Hordeum, Poa, Sitanion* and other genera is thought to be in the common heavy infestations of stem smut in quack grass, *Agropyron repens*, in eastern Washington and adjacent Oregon and Idaho.

The Blind-seed Disease of Ryegrass (Lolium spp.) in Oregon. GEORGE W. FISCHER. During 1942 and 1943 considerable difficulty was encountered with low germination of seed of perennial ryegrass, *Lolium perenne*, in sections of the Willamette Valley, Oregon. This reduced germination has now been positively identified with the blind-seed disease (*Phialea temulenta*). Apothecia have been found in abundance on *L. perenne* and *L. temulentum*, and a few on old seeds of *Hordeum gussoneanum*. The conidial stage of the blind-seed fungus was found on *Agrostis exarata* var. *monolepis, Aira caryophyllea, Alopecurus geniculatus, Bromus racemosus, Cynosurus echinatus, Deschampsia caespitosa, Danthonia californica, Festuca elatior, F. myuros, Glyceria borealis, Hordeum gusso-*

neanum, Holcus lanatus, and *Phleum pratense.* A method of qualitative and quantitative detection of blind-seed in current crops of perennial ryegrass and in seed samples is now being worked out. It is a modification of the New Zealand method in that random head samples are chopped and soaked a short time in water, which is then strained through cheesecloth and centrifuged. The residue is then examined microscopically for the presence of the characteristic conidia, the extent of whose presence indicates the severity of the disease in the field represented. No apothecia were found where low-germination (53 per cent) seed was planted in the spring, whereas the same seed, planted in the fall gave an average of 6.8 apothecia per square foot the following spring.

Vegetable Seed-treatment Trials in Western Washington in 1944. C. J. GOULD. Vegetable seed-treatment tests in southern, central, and northern areas of western Washington in the spring of 1944 demonstrated: that materials superior in one area were generally superior in all, despite differences in soil type, rainfall, etc.; that Arasan, Spergon, and Semesan could be safely used on the crops tested by applying an excess of dust to the seed, shaking, and screening off the residue, but that Cuprocide sometimes reduced stands when used in this manner; and that of the new materials tested Arasan was very effective on many vegetable seeds, Dow 5 (tetrachloro-quinone) and Dow 6B (trichloro-phenol) were promising on a few, and U. S. Rubber 604 (dichloronaphtho-quinone) was very good on those on which it was tried. Ten treatments with 5 replications of 200 seeds each were used on each crop at each location. Twenty-six of the 35 tests were statistically significant. The best results were obtained on each crop with the following materials: Peas—Spergon, Semesan, and U.S.R. 604; Beans—Spergon and Semesan; Spinach—Arasan, Cuprocide, U.S.R. 604, and 2 per cent Ceresan; Beets—2 per cent Ceresan and Arasan; Chard—2 per cent Ceresan and Arasan; Squash—Cuprocide and U.S.R. 604; Corn—Arasan and U.S.R. 604; Lettuce—Spergon, Cuprocide, and Semesan; Onion—Arasan and Semesan; and Kale—Semesan and Zinc Oxide.

Leaf Roll of Potato in Washington. LEON K. JONES. Leaf roll has become extremely damaging on potatoes in central Washington since 1938. The disease has spread very rapidly, and infection takes place from the time that plants emerge until the end of the growing season. The virus has been transmitted by grafting and by *Myzus persicae* Sulz., *Myzus pseudosolani* Theob., and *Macrosiphum solanifolii* Ashm., but not by mechanical inoculation methods. The abundant dissemination of the virus during the early season indicates that some other insect, as well as aphids, may also be a carrier of the virus. The yield of potatoes was reduced from normal to about 15 per cent when 100 per cent of planting stock was infected with the virus, to 30 per cent when 25 to 30 per cent of planting tubers were infected, and to 60 per cent of normal when 12 to 15 per cent of planting tubers carried the virus. Early current season infection reduced yields to about 65 per cent of normal and late current season infection caused little or no reduction in yield.

Incidence of Phoma Infection on Sugar-beet Seeds and the Efficiency of Seed Treatments. L. D. LEACH. Among 125 seed lots planted in pasteurized soil in the greenhouse and examined for *Phoma* infection of seedlings, 24 lots were heavily infected. Severity of infection was apparently related to the summer rainfall in the seed-producing areas, being heavy on seed from the Willamette Valley, Oregon, where overhead irrigation is practiced, light to moderate on seed from Medford, Ore., Shasta Valley, Calif., and St. George, Utah, and extremely light on seed from Hemet Valley, Calif. The practice of harvesting two successive seed crops from the same roots often resulted in several times as much *Phoma* infection on the second crop as on the first. Only certain seed lots, planted at low temperature, suffered severe pre-emergence damping-off from seed-borne *Phoma.* In germination trials with controlled soil temperatures, emergence from such lots increased with the temperature up to 25° C., above which only post-emergence infection occurred. Control, but not elimination, of seed-borne infection was obtained by dusting the seed with Ceresan, New Improved Ceresan, Arasan, or dichloro-naphthoquinone. Spergon and Yellow Cuprocide were relatively ineffective. A dip treatment with an ethyl mercury phosphate solution completely eliminated *Phoma* infection from 27 of 31 seed lots tested.

The Application of Vapor Heat as a Practical Means of Disinfecting Seeds. F. P. MCWHORTER and P. W. MILLER. The results of several hundred tests indicate that moist heat supplied by a vapor-heat machine has many advantages over hot water for seed treatment. These are: (1) critical temperature control is unnecessary to insure disinfection and prevent injury to seed; (2) treatment can readily be applied to tons of seed at a time; (3) seeds are only slightly dampened and the same machine removes the excess moisture without rehandling the seeds; and (4) vapor heat can be applied to seeds on moving belts suitable for large scale commercial seed handling. Peas, beets, cabbage, and several grasses will stand temperatures of 140° F. to 150° F. for 90 minutes to 40 minutes, respec-

tively, without significant reduction or retardation of germination. Pathogens belonging to the genera *Macrosporium*, *Fusarium*, and *Sclerotinia* are consistently killed at much lower temperatures in shorter time. *Phoma* is usually killed and bacterial infestation greatly reduced. Practical control of *Phoma* on beets has been demonstrated. Preliminary tests indicate that *Sclerotinia* as a contaminant of cabbage seed and nematodes in and on grass seeds can be completely eliminated without injury to the seeds.

Transmission Studies with Alfalfa Witches'-broom. J. D. MENZIES. Witches'-broom of alfalfa is graft-transmissible, with symptoms appearing in the stock approximately 2 months after shoot grafting. When root grafting is used, the latent period varies from 5 to 6 months. The disease has been transmitted by shoot grafting to *Medicago lupulina* and *M. hispida*. Grafts of diseased scions to species of *Melilotus* and *Trifolium* all failed to form unions, although many scions remained living for as long as three months. These scions persisted for this length of time by producing roots within the pith tissues of the stock stem. No disease transmission occurred with any of these inter-generic grafts. Witches'-broom could not be transmitted by mechanical sap inoculation nor through the use of dodder. No evidence was obtained of transmission through seed. Insect vector tests have given good evidence that the leafhopper, *Platymoideus acutus* Say., is capable of transmitting witches'-broom. This species occurs throughout the United States and Canada, indicating a danger of future spread of witches'-broom to new areas.

Studies on the Control of Bean Rust. J. A. MILBRATH. Bean rust in Oregon has become of sufficient importance to warrant control only in one area where there is a large acreage of Blue Lake pole beans. Since the uredial stage of the rust has not been found until about 3 weeks after the first appearance of the aecia, probably the telial spores are the only overwintering spores in this area. Large numbers of teliospores can be found adhering to stakes used in the bean yards. Bean yards using old stakes from rusted fields invariably have more rust infection early in the season than those fields or parts of the same field using new stakes. Wire yards usually have less rust than staked yards. When contaminated stakes were placed with beans growing in cages in the greenhouse, numerous pycnial pustules developed in 3 to 4 weeks, while unstaked plants remained free from rust. Dipping contaminated stakes in lime-sulphur 1–10 or in copper sulphate 8–100 prevented infection from the stakes. The commercial dusting sulphur, Kolodust, has greatly reduced rust infection when applied at 7-day intervals, if the dusting program was started before aeciospore formation.

A Disease of Cabbage in Western Oregon Due to Cercosporella albomaculans. P. W. MILLER and F. P. MCWHORTER. During 1943 the foliage of cabbage grown for seed in Coos and Curry counties, Oregon, was attacked by a *Cercosporella*. The disease superficially resembled downy mildew infection, young lesions being black, of dendritic form; the old spots are rounded or rectangular, 10 to 20 mm. in diameter, definitely marginated. Wild turnip, *Brassica oleracea*, occurring as a weed in the plantings was abundantly infected with *Cercospora* (*Cercosporella*) *albomaculans* Ell. et Ev. The species name refers to the white spots characteristic of the fungus on wild turnip. Cross-infections between cabbage and turnips and turnips and cabbage were readily obtained. Conidial characteristics of the fungus on both hosts place it definitely in the former genus *Cercosporella*, a genus given by Ellis and Everhart as second choice when they described the fungus from mustard. The cabbage disease is uncommon and is here interpreted as a case of natural transfer from wild turnip.

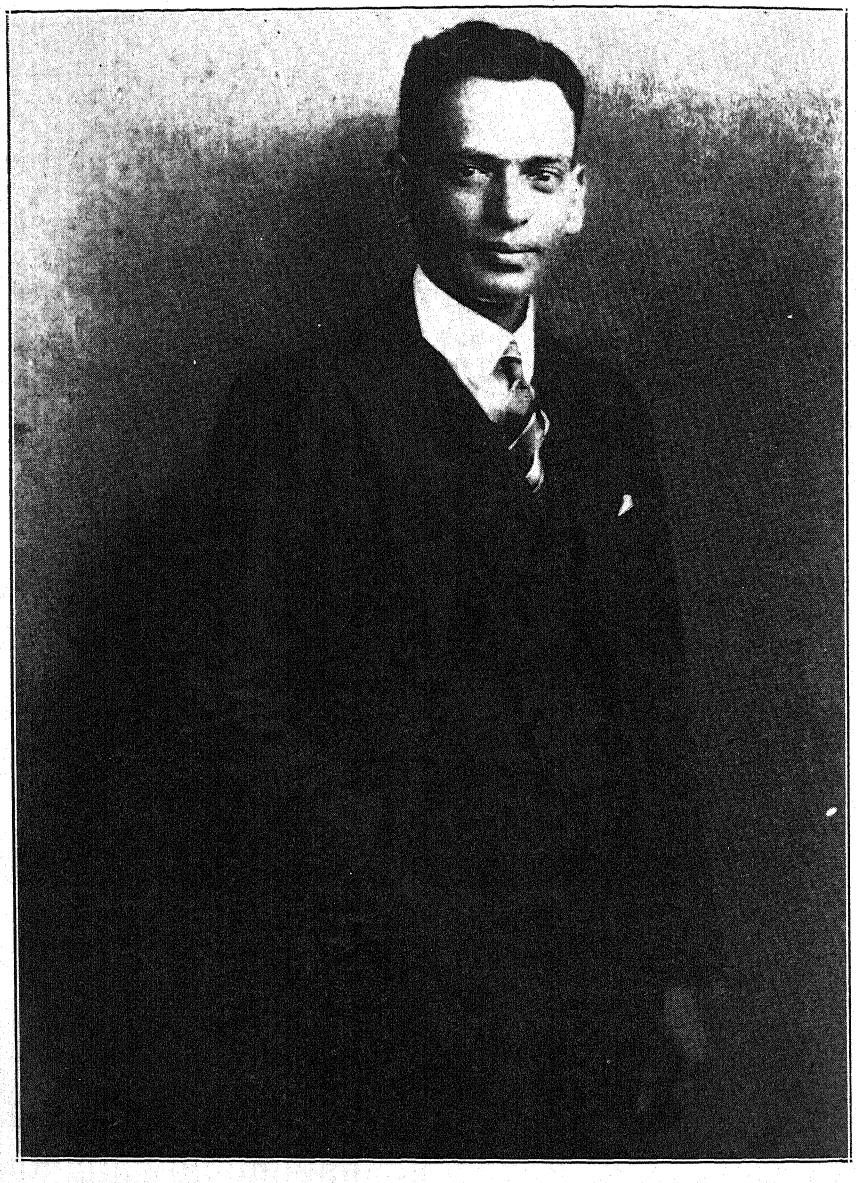
Verticillium Wilt of Guayule. HENRY SCHNEIDER. Verticillium wilt has been destructive in field-planted guayule in Kern County and less so in Monterey County, California. It has also been observed in indicator plots in Arizona and Texas. There was considerable variation in the resistance of the different guayule strains to wilt in the field: variety 405 was resistant, variety 109 was very susceptible, and varieties 406 and 593 (the ones commonly planted) were intermediate in susceptibility. In the San Joaquin Valley, Verticillium wilt was active during the spring and fall of 1943 when mean air temperatures were about 70° F. June, 1943, was unusually cool and consequently wilt was active for a longer period than usual. No new infections were noted from July through September when mean air temperatures were about 80° F. Few infections occurred in the fall of 1943 in plots kept dry. In irrigated plots the numbers of infections were inversely proportional to frequency of irrigations. Diseased plants did not recover as well in dry plots as in those that were irrigated.

Copper Sulphate as an Eradicant Spray. C. E. YARWOOD. Solutions containing 0.001 to 0.1 per cent bluestone plus 0.05 per cent spreader applied to infected plants have given fair to excellent eradication of powdery mildews on bean, cucumber, cantaloupe, and rose

without marked host injury in greenhouse and field and have increased the yields of beans, cucumbers, and cantaloupes in the field. The dosage for equivalent eradication decreased with age of infection up to about 8 days, and was less during the day than at night. In greenhouse tests about 6 times as much bluestone was required for 95 per cent eradication when equal lime-Bordeaux (equal quantities of bluestone and of lime) was used as when bluestone without lime was used, but Bordeaux had greater protective action than bluestone. This bluestone spray is considered of value in situations where sulphur dusts or sulphur sprays are unsatisfactory, and powdery mildew is the principal disease to be controlled. As an eradicant spray for bean rust and cucumber downy mildew, bluestone was more effective than Bordeaux, but was effective only up to about 24 hours after inoculation, and is not considered of practical value.

Observations on the Overwintering of Powdery Mildews. C. E. YARWOOD. Of the powdery mildews observed on 175 host species in California, perithecia have been observed on only 44. Heavy mildew infection on a few to many newly opened buds in the spring when most opening buds are healthy, indicates that bud infection may be an important means of overwintering of the powdery mildews on apple, peach, wild rose, lilac, raspberry, grape, oak, plum, cotoneaster, *Photinia serrulata, Spirea bumalda,* and *Pyrocantha crenulata.* The occurrence of conidial stages of powdery mildews on rose, cereals, crucifers, Euonymus, Hydrangea and on several weeds during the winter months indicates that these powdery mildews may overwinter as mycelium on the leaves, or as successive conidial generations. Pannose mycelium has been observed on peach and rose twigs during the winter, but one attempt to induce this mycelium to form conidia was unsuccessful. Some powdery mildews have been observed only in greenhouses. The most acceptable manner of overwintering suggested for the powdery mildews of bean and cucurbits is that they overwinter in their conidial stages in southern regions and blow north each season. This same explanation may apply to red clover mildew in Indiana, but there is circumstantial evidence of overwintering as mycelium and as bud infections.

Albino Cherry, a Virus Disease in Southern Oregon. S. M. ZELLER, J. A. MILBRATH, and C. B. CORDY. Albino cherry, apparently a type of buckskin disease, discovered in Ashland in 1937, has now spread as far as Gold Hill, Oregon. Affected Bing or Napoleon trees are usually killed within 3 or 4 years after first symptoms appear, while Lambert, Black Republican, and Montmorency varieties react more slowly. Trees whether on Mazzard or Mahaleb roots behave similarly. The disease is more severe under irrigation than under dry-land culture. All or any portion of a tree may be affected, with die-back a usual symptom of diseased branches. The leaves become a uniform olive brown to golden greenish, with margins rolled upward. Leaves about to shed become chlorotic to orange, with some pinkish tints, especially in a pinnate pattern along the midrib and lateral veins at the base. Late-summer growth of small, green, rosetted leaves is produced from terminal buds on spurs. This characteristic is general on moderately diseased trees although occasionally it is the first symptom observed on otherwise vigorous trees. Fruits remain small and green, turning white even in dark red varieties. Symptoms from inoculations have not proved satisfactory for study since affected trees die within a year after inoculation.



MANORANJAN MITRA
1895–1942

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B. B. MUNDKUR

Manoranjan Mitra, Assistant Mycologist at the Imperial Agricultural Research Institute, New Delhi, since 1921, died on the 10th of July, 1942, at his home at Hazaribagh, following diabetic coma.

Mitra was born at Amritsar on June 19, 1895, third son of a gifted family of Bengali Christians, who had settled down in the Punjab. During his boyhood he attended the Central Model School at Lahore from which he was graduated in 1912. He entered the Punjab University (Government College), Lahore, the same year and received the B.Sc. degree in 1916 and the M.Sc. degree in 1918, majoring in botany, first class, first on the roll. His M.Sc. thesis was on the anatomy and development of some Himalayan species of *Adiantum* and *Pteris*.

He served for a time as a lecturer at St. John's College, Agra, but his interests lay, however, in research and in 1919 he came to the Institute as a graduate student to get training in mycology under Sir Edwin J. Butler F.R.S. who was then the Imperial Mycologist. In 1921 the Associateship of this Institute was conferred on Mitra. As a graduate student he worked on an *Acrothecium* attacking bajra (*Pennisetum typhoides*), to accommodate which he established the species *Acrothecium penniseti*.

Soon after the completion of his training, Mitra was appointed Assistant Mycologist, a post that he held until his death.

In 1926 he was deputed by the Government of India to survey the crop diseases occurring in the Andaman Islands and in 1927 he was granted sabbatical leave to proceed to England. He joined the Imperial College of Science, London University, where his investigations were mainly on the species of *Helminthosporium* affecting Indian cereals. He was granted the diploma of the Imperial College in 1929 and the Ph.D. degree of the London University the same year, and that of D.Sc., in 1936.

Mitra's chief interest lay in the diseases of cereals, especially those caused by species of *Helminthosporium*. The earlier accounts of this genus failed to distinguish the Indian species correctly but as a result of Mitra's investigations, our knowledge of this genus is now on a stable basis. In 1930 he discovered a bunt of wheat in the plains of India, now known as the "Karnal Bunt," and named the fungus *Tilletia indica*. He has two more species to his credit, *Helminthosporium bicolor* and *Helminthosporium frumentacei* and a variety, *Helminthosporium halodes* var. *tritici*.

Mitra was a man of retiring nature; he felt more at home among his fungi and his books than elsewhere. His knowledge of Indian plant diseases was very profound; and the graduate students of the mycology section found in him a scholarly teacher. He was a member of the Linnean Society, British Mycological Society, The American Phytopathological Society and

the Indian Botanical Society. He leaves his wife and two sons and a large circle of friends to mourn his loss.

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STREAK AND MOSAIC OF CINERARIA[1]

LEON K. JONES

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INTRODUCTION

During the winter of 1937–38, a disease of cineraria was noted in one greenhouse in Spokane, Washington. Necrosis of leaves and stems often developed to such an extent that most of the leaves on affected plants died progressively up the stem and many plants wilted and died previous to, and during, blossoming. The disease was called streak[2] and was investigated in the Experiment Station greenhouses at Pullman, Washington, and in commercial greenhouses in various localities in the State to determine its nature and the methods of control. A disease of cinerarias with symptoms similar to those associated with streak was reported in 1934[3] as caused by the spotted wilt virus. The present studies included tests to determine the possible relationships of the 2 diseases.

In a greenhouse in Walla Walla, Washington, in January, 1938, 2 per cent of the cineraria plants were affected with a typical mosaic disease. The leaves were mottled with large, raised, dark green areas in the otherwise light green tissue and were puckered and malformed, and the plants were somewhat dwarfed. In the literature one reference to a mosaic disease of cineraria was noted.[4] The briefness of this reference makes it impossible to determine the exact nature of the mosaic-like trouble or its possible relationship to the mosaic disease observed in Washington. Lettuce mosaic has been transmitted to groundsel (*Senecio vulgaris* L.).[5] Groundsel is very closely related to cineraria and cinerarias are listed by some taxonomists as a development out of *Senecio cruentus* D. C. Other taxonomists prefer to classify cinerarias under the binomial, *Cineraria cruenta* Mass. Since lettuce mosaic produces a mild mottle on groundsel, these investigations included tests to determine if cineraria mosaic was caused by the lettuce mosaic virus which is commonly transmitted in lettuce seed. Further studies were made to determine the nature of the cineraria mosaic virus and measures of value in reducing losses from this disease.

SYMPTOMS

Streak

In commercial stock, the disease ordinarily does not become destructive until the plants approach blossoming, although the ruffling and rugosity of

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[2] Washington Agricultural Experiment Station Annual Reports 49: 61. 1939; 50: 77. 1940; 51: 84. 1941; and 52: 77–78. 1942.

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leaves of younger plants indicate infection (Fig. 1, A). Rugosity and curling of leaves of young plants are very evident as the first symptoms following artificial inoculation. Reddish-brown areas in leaf veins (Fig. 2, C), petioles, and stalks of middle-aged plants may be observed with strong light transmitted through those areas. These areas may enlarge and constrict the affected tissues, which leads to necrosis of large triangular areas in the leaves (Fig. 3, B), necrosis of entire leaves (Fig. 4), or death of the plant. Wilting, rolling, yellowing, and death of leaves (Fig. 4) are commonly noted as the affected plants approach blossoming. Under some conditions, numerous, small, brown necrotic spots in the leaves are associated with the trouble

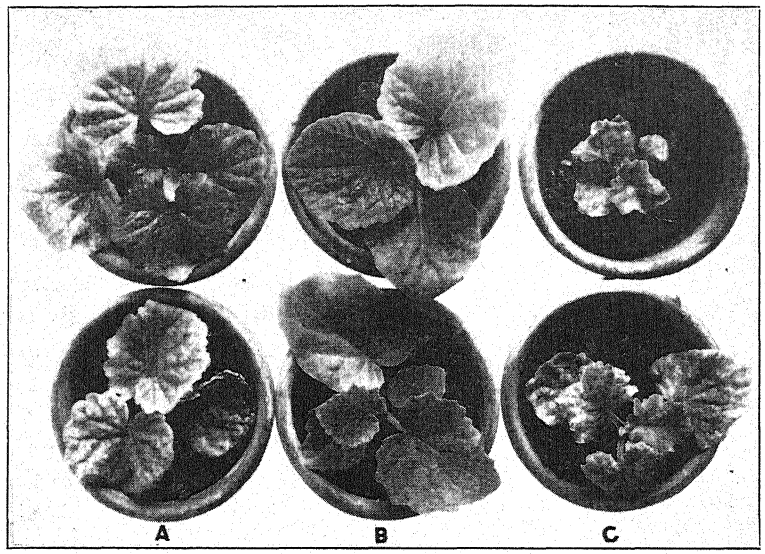


FIG. 1. Cineraria seedlings showing symptoms of streak (A), and streak and mosaic (C), compared with healthy seedlings (B). The plants became infected with the viruses by seed transmission.

(Fig. 3, A). The necrosis often develops to such an extent that most of the leaves die progressively up the stem and very few leaves are left on the plant at blossoming time. Affected plants wilt more easily than healthy plants and do not recover from wilting as readily.

Mosaic

The disease shows as mottling of the foliage with irregular light and dark green areas (Fig. 2, A and B). Crinkling, ruffling and dwarfing of leaves (Fig. 2, A), as well as shortening of leaf petioles and dwarfing of plants, are symptoms commonly associated with mosaic. Vein-clearing and puckering of young leaves following inoculation by mechanical methods precedes the appearance of the typical mottling.

Streak and Mosaic

The 2 diseases are often noted in the same plant, and a combination of

symptoms appears (Fig. 1, C). Plants are more severely dwarfed than are plants affected with either disease alone. Necrosis often appears in the light areas of the mosaic pattern in the middle-aged leaves before yellowing and necrosis appear in the older leaves.

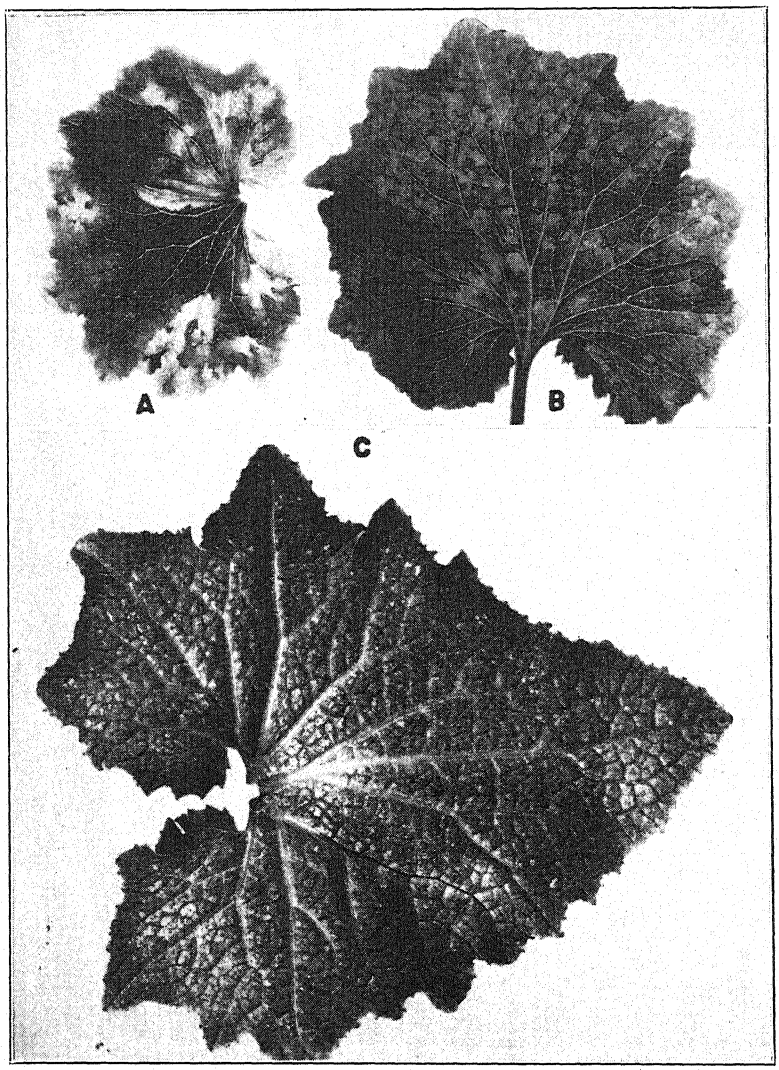


FIG. 2. Leaves of cineraria with mosaic and streak symptoms. A, ruffling, rugosity, and mottling on young leaf, and B, mild mottling of older leaf caused by the mosaic virus. C, necrosis of leaf vein caused by the streak virus.

ECONOMIC IMPORTANCE

Streak

In the greenhouse where streak was first noted, 1200 to 1500 cinerarias are grown each season. The grower estimated that one-half of his plants in

1936, and possibly one-third in 1937 and in 1938, were discarded because of the presence of streak. Many diseased cinerarias were exhibited by growers in plant clinics held in Seattle, Washington, in 1939, and Spokane, Washington, in 1940, at conventions of the Northwest Florists, and the opinion was expressed that it was a serious problem that had developed only during the preceding few years.

Observations in greenhouses in Washington from 1938 through 1942 have shown that streak was usually present in the cineraria crop with the exception of crops produced by a few growers who saved seed from their own plant selections (Table 1). Counts were made to determine the prevalence

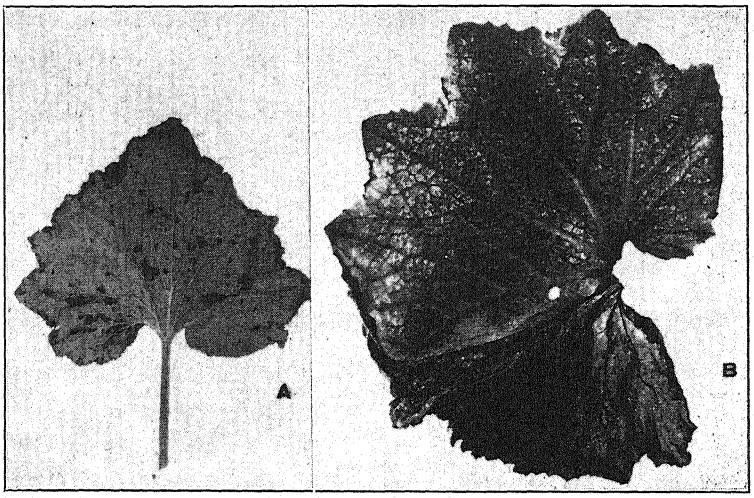


FIG. 3. Necrosis of cineraria leaves caused by the streak virus. A, scattered, brown necrotic spots and B, large necrotic area following vein necrosis.

of streak in the stock, but it was not possible to obtain data on the losses caused by the disease beyond determining the percentage of plants affected. Probably a high percentage of the affected plants was unsalable. The sale of affected plants also leads to an unfavorable reaction by the buying public, since such plants ordinarily have very poor keeping qualities when transferred from the humid greenhouses to the relatively dry atmosphere of residences.

Mosaic

In a greenhouse in Walla Walla, Washington, in January, 1938, about 2 per cent of the cineraria plants observed were affected with mosaic. The disease is not very prevalent, but is quite generally distributed (Table 1) since it was observed in Spokane, Walla Walla, Seattle, and Kent, Washington. Severely affected plants may be sufficiently dwarfed to be unsalable but, in general, the disease does not materially affect the salability of plants.

ETIOLOGY

The mottling-type of disease appeared very definitely to be a typical

mosaic caused by a virus. The streak disease, however, showed symptoms that are often associated with disease caused by parasitic organisms. Forty-four attempts to isolate an organism from necrotic areas in petioles, stems, and leaves of streak-affected plants were made in January and February of 1938. No fungi appeared on agar plates and bacteria occurred in only 3 instances. The failure to isolate an organism consistently from diseased tissue indicated that a virus was probably responsible for the trouble.

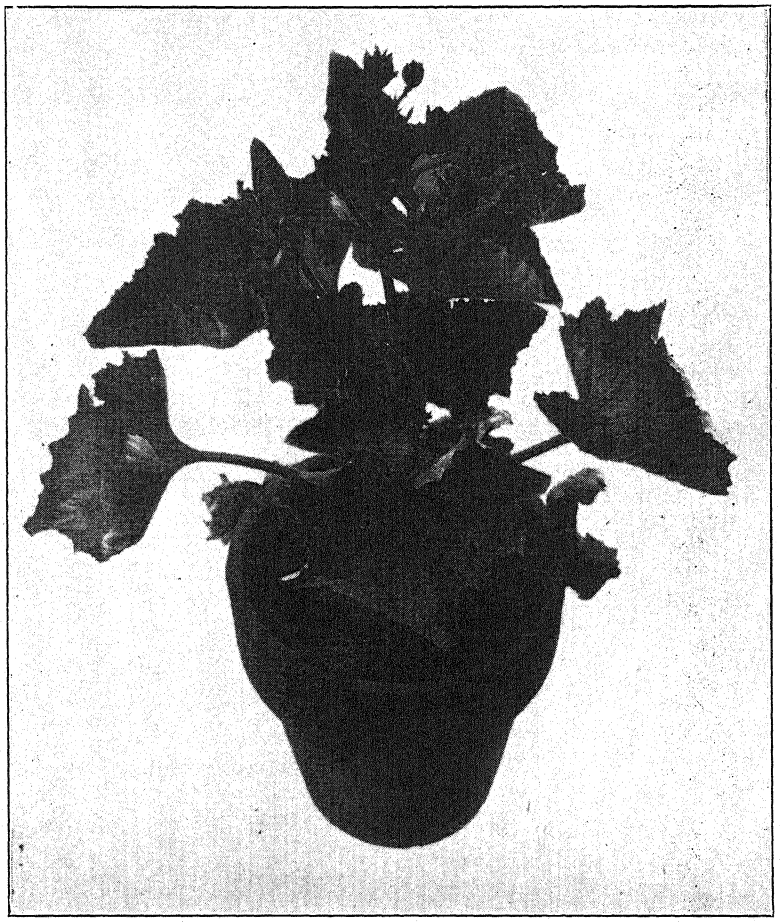


FIG. 4. Rolling, wilting, and death of cineraria leaves following inoculation with the streak virus.

Inoculations, by mechanical transfer of the virus, were made from diseased plants to healthy plants to determine if viruses were responsible for mosaic and streak. In 3 tests, 40, 50 and 82 per cent of the mosaic inoculations were successful, with an incubation period of 20 to 24 days. Sixteen, 50 and 50 per cent of the streak inoculations were successful, with an incubation period of 14 to 35 days. Since these preliminary tests showed that both streak and mosaic were caused by viruses, detailed studies were made to determine the characteristics of the viruses.

SEED TRANSMISSION

Some strains of cineraria stock in greenhouses in Spokane, Walla Walla, and Seattle in 1938 and 1939 were severely affected with streak or mosaic,

TABLE 1.—*The prevalence of streak and mosaic in cineraria plants in greenhouses of Washington*

Greenhouse	Year observed	Source of seed[a]	No. plants grown	Percentage[b] of plants with symptoms of	
				Streak	Mosaic
1	1938–1939	Local	1000	50	0
1	" "	Commercial	500	10	0
2	" "	"	800	0	5
3	" "	Local	800	0	0
4	" "	"	1000	20	2
5	" "	"	1500	0	0
5	" "	Commercial	400	50	10
6	" "	"	900	1	0
7	" "	"	600	50	0
8	" "	Local	1600	0	0
2	1939–1940	Commercial	800	20	0
9	" "	"	1200	20	0
1	" "	Local	1000	40	0
10	" "	"	1200	20	0
1	1940–1941	"	1200	25	0
1	" "	Select	500	0	0
10	" "	Local	800	75	0
11	" "	Commercial	300	50	0
12	" "	"	650	50	0
13	" "	"	900	75	0
14	" "	"	500	50	0
6	" "	"	1200	5	2
15	1941–1942	Local	800	0	0
15	" "	Commercial	1000	40	0
16	" "	"	300	20	0
8	" "	Local	1000	0	0
8	" "	Select	500	0	0
1	" "	Local	1200	10	0
1	" "	Select	600	0	0
5	" "	"	1500	0	0
10	" "	"	800	0	0
10	" "	Commercial	500	15	1
9	" "	"	500	20	2
9	" "	Select	600	0	0
17	" "	Commercial	950	75	0
18	1942–1943	Select	100	0	0
8	" "	"	800	0	0
10	" "	"	500	0	0
7	" "	"	500	48	0
17	" "	"	600	65	0
17	" "	Commercial	400	68	0
1	" "	Local	1000	52	0

[a] Local—seed collected by local growers; Commercial—seed purchased from commercial seed companies; Select—seed selected from virus-free plants at the State College of Washington.

[b] Percentages—based on random counts of at least two 100-plant lots in each crop observed during the months of January to March.

while other strains in the same greenhouse were free of these diseases. A strain of cineraria developed by one grower had considerable streak each season, not only on plants grown by the originator, but also on plants grown

from the same seed in 2 other greenhouses. Three strains of cineraria grown by local growers from seed of their own selection were free of streak in 1938–1939, although one strain had a low percentage of mosaic. Stock grown in a number of greenhouses from seed purchased from various seedsmen (Table 1) usually had varying percentages of streak, and stock from 5 of 16 commercial-seed strains had low percentages of mosaic. These observations indicated that each virus might be transmitted in the seed. Accordingly, several samples of seed were obtained from leading seed companies and local growers for virus-transmission tests in the greenhouses at Pullman, Washington (Table 2). Seed was also collected from mosaic- or streak-affected plants and tested to determine if the viruses were carried in the seed (Table 3).

TABLE 2.—*The transmission of mosaic and streak viruses in commercial seed. Pullman, Washington. 1938–1942*

Lot, year and variety of seed tested	Source of seed	No. plants grown	Percentage of plants with symptoms of		
			Mosaic	Streak	Mosaic and streak
1938–1939					
1 Grandiflora Nana	Seedsman No. 1	178	24	69	7
2 Howard & Smith	" " "	44	70	0	0
3 Prize mixed	" " "	129	5	20	2
4 Prize	Seedsman No. 2	130	9	32	5
5 Siter's Rainbow	Seedsman No. 3	70	0	57	0
6 Multiflora Nana	" " "	270	0	26	0
7 Hybrida Grandiflora	Local grower	53	0	47	0
1939–1940					
3 Prize mixed	Seedsman No. 1	36	0	42	0
4 Prize	Seedsman No. 2	139	0	35	0
5 Siter's Rainbow	Seedsman No. 3	146	0	34	0
7 Hybrida Grandiflora	Local grower	100	0	30	0
1941–1942					
8 Improved Prize	Seedsman No. 2	234	0	10	0
9 Prize, reselected	Seedsman No. 4	235	0	17	0
10 Hybrida mixed	" " "	117	0	14	0
11 Hybrida Grandiflora	" " "	207	0	20	0
7 Hybrida Grandiflora	Local grower	234	0	20	0

Each lot of seed was planted separately in flats and handled independently during potting. The hands were washed thoroughly with soap and water previous to handling each lot of plants. Where sufficient seed was available, a portion was used in 1938 and the remainder in 1939, except that the seed in lot 7 from a local grower was used again in 1941–1942 (Table 2).

The seed was planted in flats during August each season. The young plants were placed in 2½-inch pots in September and repotted to 4-inch pots in December. Plants with definite mottle symptoms of mosaic, or necrosis of streak, were discarded from each lot as soon as observed. Aphids were controlled as soon as observed by fumigation with nico-fume powder, but no attempt was made to control thrips. Accordingly, the percentages of plants listed in Tables 2 and 3 as showing symptoms of mosaic probably give a true

indication of the seed transmission of this virus. The percentages of plants with symptoms of streak, however, probably include considerable current season infection from a lower percentage of plants originally affected from seed transmission of the virus. Ruffling and rugosity of the foliage of young plants affected with streak indicates the presence of this virus, but this symptom alone was not considered sufficiently definite to discard plants. The

TABLE 3.—*The transmission of mosaic and streak in locally selected seed. Pullman, Washington. 1940–1942*

Plants from which seed was selected and year tested	Diseased condition of seed plant[a]	No. plants grown	Percentage of plants with symptoms of	
			Mosaic	Streak
1940–1941				
1	Streak	212	0	64
2	"	95	0	91
3	"	37	0	92
4	"	34	0	94
5	"	82	0	83
6	"	50	0	94
7	"	98	0	88
1–7 mixed	"	110	0	96
8	Mosaic and streak	240	20	79
9–13 mixed	Virus-free	49	0	0
17	Mosaic	250	19	0
18	Mosaic and streak	83	35	27
19	" " "	50	24	10
20	" " "	61	100	50
9	Virus-free	200	0	0
10	" "	144	0	0
11	" "	156	0	0
12	" "	120	0	0
13	" "	42	0	0
14	" "	46	0	0
14–16 mixed	" "	220	0	0
1941–1942				
1	Virus-free	180	0	0
2	" "	225	0	0
3	" "	234	0	0
4	" "	234	0	0
5	Streak (1939)	234	0	20
6	" (1941)	234	0	2
7	" "	234	0	30
8	Streak and mosaic (1941)	210	23	11
9	Streak (1941)	117	0	12
10	Mosaic (1940)	350	0	0
11	Virus-free	234	0	0
12	" "	234	0	0

[a] Seed collected the previous season unless otherwise noted.

plants were kept until definite necrotic symptoms appeared and there may have been some opportunity for spread of the virus.

All commercial lots of seed tested carried one virus and some lots carried both viruses. Often both viruses were carried in individual seeds. The streak virus remained active in four-year-old seed, but the mosaic virus was inactivated by one year of storage in seed lots 3 and 4 of commercial seed. In further tests with seed collected at Pullman, Washington, from mosaic-

affected plants (Table 3, plants 8 and 11 in 1941–1942), the mosaic virus was not inactivated in one-year-old seed.

The tests with locally selected seed showed that virus-free seed could be obtained by carefully selecting symptomless plants for seed production (Tables 1 and 3). In 1942–1943, however, selected seed failed to produce healthy plants in greenhouses 7 and 17. Weeds under the benches in these greenhouses appeared to be responsible for the carry-over of the virus from one season to the next. Definite symptoms of spotted wilt were observed on prickly lettuce (*Lactuca scariola*) under the benches in greenhouse 7. Symptoms of a possible virus disease on wood sorrel (*Oxalis pumila*) under benches in greenhouses 7 and 17 also indicated that this weed might be responsible for the carry-over of the virus which could be transmitted readily by thrips to the new crop of cineraria.

MECHANICAL TRANSMISSION

Preliminary tests in 1938–1939 showed that both the streak and mosaic viruses could be successfully transmitted by mechanical inoculation methods.

TABLE 4.—*The effect of extraction of plant juice upon the activity of the streak and mosaic viruses*

Virus used	Method of inoculation	Results obtained in different series of inoculations[a]	Period of incubation
			Days
Streak	Rubbed leaf tissue	7/20, 8/10, 12/20, 8/10, 3/5	14–35
Streak	Juice extract	0/40, 0/20, 0/20[b]	.. ..
Mosaic	Rubbed leaf tissue	6/10, 14/20	25–42
Mosaic	Juice extract	13/20	35–42
Controls	No treatment	0/40, 0/10, 0/40, 0/40	.....

[a] Each fraction represents a series of inoculations, the numerator shows the number of plants infected and the denominator the number of plants inoculated.

[b] Ten cc. of one-half per cent sodium sulphite was added to 100 grams of frozen leaf tissue previous to extraction of juice in press.

Forty to 82 per cent of the mosaic inoculations and 16 to 50 per cent of the streak inoculations were successful. In these tests portions of the diseased leaf were wrapped around a cotton swab and slightly macerated on a pot label before being rubbed over the foliage of the plants to be inoculated. Carborundum dust was sprinkled over the leaves to be inoculated previous to rubbing the diseased tissue upon them.

In later inoculation tests, with 2 other methods of inoculation based on the extraction of the plant juice from the leaves before rubbing onto healthy foliage, the streak virus was not transmitted (Table 4). These methods included macerating the affected leaves in a mortar, filtering through cheesecloth or freezing the affected leaves for 12 hours before extracting the juice in a hydraulic press. In each case the juice was diluted one to 10 with distilled water before being rubbed onto leaves dusted with carborundum. The mosaic virus was transmitted equally well by all of the inoculation methods used (Table 4). The streak virus was inactivated very quickly in

extracted plant juice, and accordingly, the handling of plants in ordinary cultural practices probably would not account for much transmission of the disease. The relative ease of transmitting the mosaic virus in extracted juice indicated that the handling of plants in routine cultural practices might account for considerable transmission of the disease.

INSECT TRANSMISSION

Two insects, *Aphis marutae* Pestlund[6] and *Thrips tabaci* Lindeman, were commonly observed on cineraria plants. These insects were transferred to healthy plants under controlled conditions to determine if they would transmit the viruses (Table 5). The mosaic virus was transmitted by *Aphis marutae* and the streak virus was transmitted by *Thrips tabaci*.

TABLE 5.—*Insect transmission of the mosaic and streak viruses*

Source of virus	Insect tested	Results obtained in different tests[a]
Cineraria streak	*Aphis marutae*	0/6, 0/27, 0/10
Cineraria streak	*Thrips tabaci*	5/5, 12/15
Control	No treatment	0/6, 0/30, 0/10, 0/5, 0/15
Cineraria mosaic	*Aphis marutae*	3/5, 3/5, 6/10, 3/10, 19/37
Cineraria mosaic	*Thrips tabaci*	0/20, 0/5
Controls	No treatment	0/9, 0/5, 0/4, 0/10, 0/25

[a] Each fraction represents a series of inoculations, the numerator shows the number of plants infected, the denominator shows the number of plants inoculated.

THERMAL INACTIVATION AND LONGEVITY

The streak virus was inactivated by extraction from plant tissue in a number of inoculation tests. The thermal inactivation point of the virus could not be determined. It has been reported that the use of sodium sulphite in preparing an extract of chrysanthemum facilitated the detection of the spotted wilt virus in that host.[7] The use of 0.5 per cent solution of anhydrous sodium sulphite in preparing extract of cineraria leaves failed to increase the longevity of the streak virus.

The mosaic virus (Table 6) was inactivated by 10-minute exposure at a point near 70 degrees C., and it remained active in extracted juice for 14 days but not for 100 days.

HOST RANGE

A number of different crops were inoculated to determine the possible relationship of the cineraria viruses to other known viruses (Table 7). The streak virus proved to have many characters associated with the spotted wilt virus, and tomato and pea were inoculated to ascertain if these two viruses were the same. The mosaic virus was compared with pea mosaic as well as being tested on tobacco, tomato, cucumber, and pea. The results of cross

[6] Determination kindly made by Doctor George F. Knowlton, Utah State Agricultural College, Logan, Utah.

[7] Ainsworth, G. Detection of spotted wilt virus in chrysanthemum. Nature, 137: 868. 1936.

TABLE 6.—*The thermal inactivation point and longevity in vitro of the cineraria mosaic virus*

Treatment of inoculum[a]	Results of inoculations[b]
Leaf tissue	7/10, 6/10, 12/18
Fresh extract	10/20, 6/20, 12/18
Extract—50° C.	3/20, 5/20, 11/18
" —60° C.	6/20, 0/20, 8/18
" —70° C.	5/20, 0/20, 0/18
" —80° C.	0/20
Extract after inoculation	9/20, 5/20
" " 24 hours	13/18
" " 5 days	9/20
" " 10 days	2/20
" " 14 days	8/20
" " 100 days	0/20
Control	0/20, 0/20, 0/36

[a] Extracted juice was exposed to temperatures for 10 minutes.
[b] Each fraction represents a series of inoculations, the numerator shows the number of plants infected, the denominator shows the number of plants inoculated.

inoculations indicate that the mosaic virus is specific to cineraria, because it did not infect any of the other hosts tested. Comparison of the cineraria mosaic virus and the lettuce mosaic virus failed to show any relationship between these two viruses. The streak virus was successfully transmitted to tomato and pea, and the symptoms produced on these hosts further indicated a very close relationship of this virus to the tomato spotted-wilt virus. Tomato plants with symptoms commonly associated with infection by the spotted-wilt virus were used to inoculate cineraria. The symptoms produced on cineraria were similar to those associated with infection by the streak virus. These cross-inoculation tests and other characters, such as rapid

TABLE 7.—*The host range of the cineraria mosaic and streak viruses*

Source of virus and host inoculated	Results obtained in different series of inoculations[a]
Mosaic virus from cineraria:	
Cineraria	33/40, 6/8
Tobacco	0/25
Tomato	0/34, 0/29, 0/10, 0/30
Cucumber	0/10
Lettuce	0/20
Pea	0/12
Streak virus from cineraria:	
Cineraria	1/6, 5/10, 20/40
Tomato	3/30, 4/25
Pea	3/20
Spotted wilt virus from tomato:	
Cineraria	6/20, 6/24
Lettuce mosaic virus from lettuce:	
Cineraria	0/8, 0/14
Lettuce	11/20

[a] All inoculations made by rubbing macerated leaf tissue on plants previously dusted with carborundum. The numerator denotes the number of plants infected and the denominator the number of plants inoculated. In each series a like number of plants were left untreated as controls. No disease was noted in the control plants.

inactivation *in vitro* and transmission by *Thrips tabaci*, show that the two viruses are the same or that the streak virus is a variant of the spotted-wilt virus. The host range of the tomato spotted-wilt virus is very large, the disease having been reported on 84 species in 19 families of the plant kingdom.[8] Since the cineraria streak virus and the tomato spotted-wilt virus are similar in nature, it may be assumed that the former has a host range quite similar to the latter.

CONTROL

Streak and mosaic can be controlled by carefully selecting disease-free plants for seed production. Selected seed was furnished to 3 growers in 1940–1941, 5 growers in 1941–1942, and 6 growers in 1942–1943. In each case in the first 2 years of tests the selected seed produced mosaic- and streak-free stock. In 1942–1943, however, streak developed in two of the greenhouses on plants produced from selected seed. These results indicated the importance of weeds in the carry-over of the virus from one cineraria crop to another and accordingly the destruction of weeds in the greenhouse may be an important control measure. In 1941–1942, two of the growers produced plants from both selected and non-selected seed. In each case streak was evident in the plants from the non-selected seed and was absent in plants from the selected seed. Observations indicate that thrips spread the streak virus very rapidly from plant to plant, so that an excess of seed plants should be selected when the plants are about half grown and further selection from this group should be made as the plants mature. The control of thrips on cinerarias should be of value in reducing the spread of streak. Selected plants should be moved as far as possible from other cinerarias.

In two greenhouses there has been severe spotted-wilt disease on tomato. This could be traced to streak-affected cinerarias in the vicinity of the tomatoes, hence it is not advisable to grow streak-affected cinerarias near tomatoes.

Measures of value in controlling mosaic, beyond the use of disease-free seed, include aphid control and the destruction of affected plants whenever noted. Since the mosaic virus is readily transmitted in plant juice and by mechanical injury of plants, care should be used not to handle mosaic-affected plants in routine cultural practices.

SUMMARY

Two virus diseases, streak and mosaic, have been destructive on cineraria in greenhouses of Washington.

Necrosis of leaves and stems and wilting of plants near blossoming time caused by the streak virus often resulted in a loss of 20 to 50 per cent of the plants.

Mottling of the leaves with light and dark green areas, puckering and malformation of the leaves, and dwarfing of plants caused by the mosaic virus in a few cases resulted in the loss of a low percentage of the plants.

[8] Holmes, Francis O. Handbook of phytopathogenic viruses. 221 pp. Burgess Publishing Company. Minneapolis, Minnesota. 1939.

The streak virus is transmitted in seed, by mechanical rubbing of diseased tissue on healthy tissue, and by *Thrips tabaci*, but is inactivated very quickly in extracted plant juice. The streak virus was transmitted to tomato and pea and the symptoms produced on these suscepts as well as the other characters of the virus show that it is a strain of the tomato spotted-wilt virus.

The mosaic virus is transmitted in seed, by mechanical inoculation methods, and by *Aphis marutae*. This virus is inactivated near 70 degrees C. and remains active in extracted plant juice for 14 days. The mosaic virus was not transmitted to tobacco, tomato, cucumber, lettuce, and pea; and accordingly, it appears to be specific to cineraria.

Streak and mosaic can be controlled by carefully selecting disease-free plants for seed production. The control of aphids and thrips on cineraria plants would aid in reducing spread of the mosaic and streak viruses. The destruction of weeds, that may be hosts of the streak virus, in the vicinity of cineraria plants and care in not handling mosaic plants previous to handling healthy plants are cultural practices of value in reducing losses.

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COMPARISON OF SOIL FUMIGANTS FOR THE CONTROL OF THE ROOT-KNOT NEMATODE

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During the past five years chloropicrin has been the outstanding soil fumigant for root-knot-nematode control. However, because it is disagreeable to work with and relatively high priced, there is need for a chemical which does not possess these disadvantages. With this in mind a number of chemicals which showed promise on the basis of small-scale greenhouse tests were compared with chloropicrin for the control of nematodes on tomatoes grown in two commercial greenhouses, one growing a spring crop and the other a fall crop.

LITERATURE REVIEW

The literature on the effectiveness of chloropicrin for nematode control was recently reviewed by Taylor (8), who reported excellent results with it at 200 pounds per acre. Several workers have obtained satisfactory nematode control with ethylene dichloride, both alone and when mixed with chloropicrin (2, 4, 6). Methyl bromide, an industrial fumigant, has been used as a soil fumigant against insect pests, and in 1940 Taylor and McBeth (9) reported good nematode control with it, using 80 cc. of the chemical per cubic meter of soil. Good results have since been reported by Taylor and McBeth (10), Gingrich and Haenseler (3), and Godfrey and Young (4). These workers all attempted to use undiluted methyl bromide, which necessitates either keeping the chemical below its boiling temperature of 40° F. or using special apparatus for measuring and dispensing it. The use of D–D mixture,[1] a crude by-product from the manufacture of allyl alcohol, containing approximately equal parts of 1,2-dichloropropane and 1,3-dichloropropylene, was first reported by Carter (1), who used it successfully against *Heterodera marioni* in Hawaii. Pinckard (7) also endorsed D–D mixture as a nemacide.

TREATMENTS FOR SPRING TOMATO CROP

The first experiments were in a commercial greenhouse of 10,000 square feet on sandy loam heavily infested with root-knot nematode in Auburn, N. Y.[2] One section of 2,000 square feet of ground bed was divided into 20 plots. The soil was thoroughly prepared with a power-driven rotary tiller. The liquid fumigants were applied with hand-operated injectors, 4 to 5 inches deep, at 10-inch intervals, in rows 10 inches apart. Four replicate randomized plots were treated with each of the following four chemicals and four were left untreated. Chloropicrin,[3] at 2 cc. per injection, was applied

[1] Supplied by the Shell Development Co., Emeryville, California.

[2] Acknowledgment is made to Dickman Brothers for their wholehearted cooperation.

[3] The chloropicrin was supplied by Innis, Speiden and Co., 117 Liberty Street, New York, as "Larvacide."

August 26, at which time many root galls from the previous tomato crop had not yet thoroughly rotted. One week later ethylene dichloride was applied at 15 cc. per injection, and a 10 per cent methyl bromide mixture,[4] at 5 cc. per injection. This methyl bromide mixture containing 67 per cent ethylene dichloride and 23 per cent carbon tetrachloride, which diluents help to keep the methyl bromide in solution, can be used in ordinary applicators. The furyl-nitro-ethylene dust[5] was broadcast at 2 pounds per 100 square feet and was then promptly forked under. After all treatments the soil was leveled by raking and was watered lightly.

After treatment in the fall of 1942 a succession of two or three crops of beet greens was grown. Examination of the roots of the first beet crop grown while the soil temperature was still fairly high gave some indication of the degree of nematode control. Plants from all plots showed some nematode injury. The methyl bromide mixture gave the best control, the chloropicrin and ethylene dichloride being almost as effective. Control in the dust-treated plots was little better than in the untreated ones in which nematode damage was severe. Subsequent beet crops were grown at such low temperatures that no injury occurred.

In March, 1943, another section of 2,000 square feet was divided into 20 plots and treated with the fumigants listed in table 2: namely, chloropicrin, chloropicrin ethylene dichloride (1–9) mixture, chloropicrin methyl bromide (3–1) mixture, and the 10 per cent methyl bromide mixture. This section of the greenhouse had been treated the previous fall with chloropicrin but, since the beet crop indicated that complete nematode control was not obtained, it was decided to re-treat before the spring tomato crop was planted. The soil was mechanically tilled before and was watered immediately after the treatments were made. In order to establish the optimum dosage of the 10 per cent methyl bromide mixture, treatments of 3, 5, and 7 cc. per injection were compared in a third 2,000-square-foot section of the house. Each treatment was replicated four times. Also, in a fourth section, dosage rates of 4, 5, 6, and 7 cc. per injection were employed, each treatment being replicated three times. These two sections had been treated in the fall of 1942, one-half of each with chloropicrin and one-half with furyl-nitro-ethylene dust, with apparently unsatisfactory results. In April the entire greenhouse was planted with tomatoes, variety Break O'Day. Records were kept on the weight and number of ripe fruits obtained from each.

At the end of the growing season the plants were all pulled and their roots examined and scored to indicate the degree of nematode injury. The method of scoring, previously described (6), was to select as standards five individual plants showing various degrees of nematode injury (Fig. 1, A), ranging from no infection, designated zero, to very severe infection, as-

[4] The ethylene dichloride and the 10 per cent methyl bromide mixture (Dowfume Br. 10) were supplied by Dow Chemical Co., Midland, Michigan.

[5] The furyl-nitro-ethylene dust, supplied by Grasselli Chemicals Division of Du Pont, Wilmington, Del., as compound No. IN2391A_2, contained 50 per cent of the active ingredient.

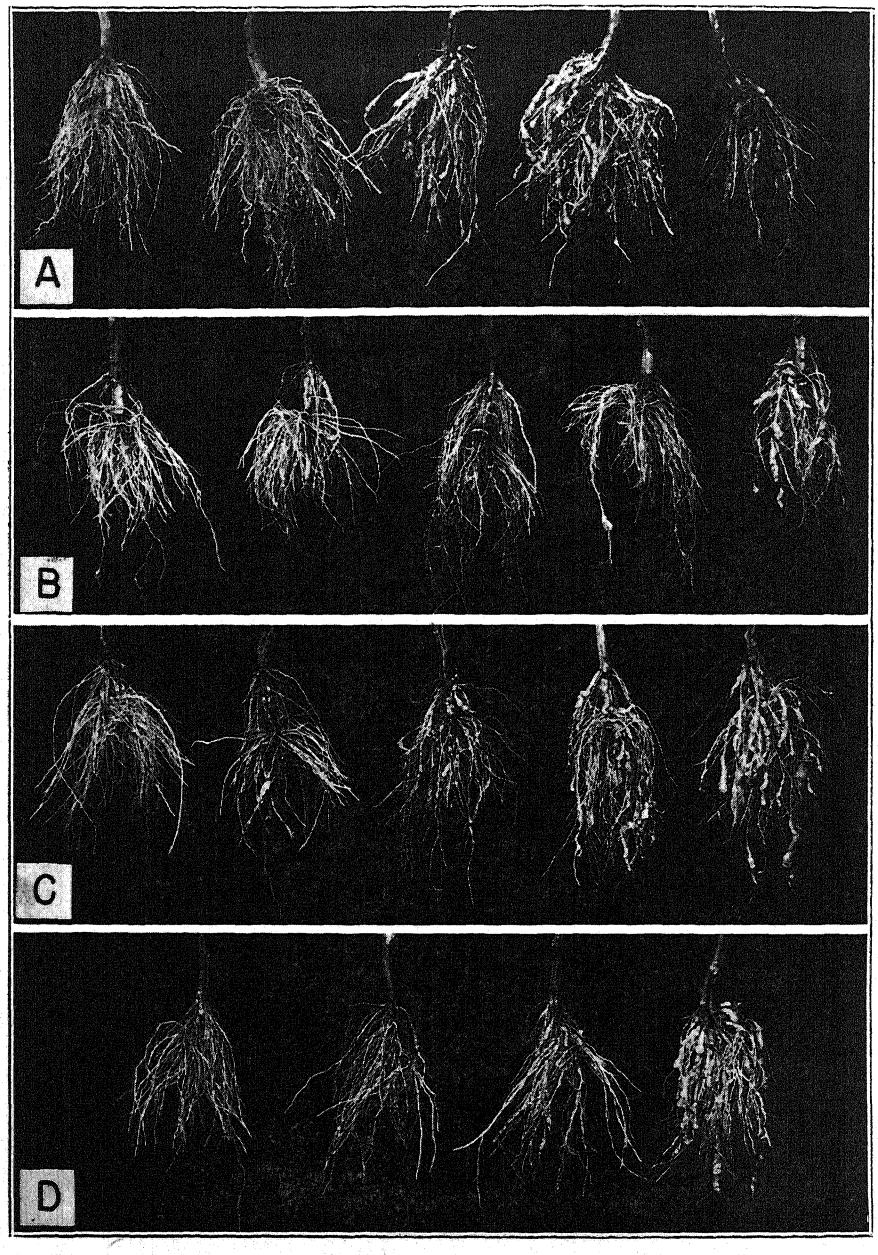


FIG. 1. A. Roots selected from spring crop as standards by which to score those from the experimental plots. They represent (left to right) classes 0, 1, 2, 3, and 4. B. Representative roots from plots receiving (left to right) 10 per cent methyl bromide mixture, 5 cc.; ethylene dichloride, 15 cc.; chloropicrin, 2 cc.; furyl–nitro–ethylene dust (2 lb. per 100 sq. ft.); and no treatment. C. Roots from plots receiving (left to right) chloropicrin–methyl bromide (3–1) mixture, 2 cc.; chloropicrin, 2 cc.; 10 per cent methyl bromide mixture, 5 cc.; chloropicrin–ethylene dichloride (1–9) mixture, 10 cc.; and no treatment. D. Roots from plots receiving 10 per cent methyl bromide treatment at (left to right) 7 cc., 5 cc., 3 cc., and no treatment.

signed a value of 4. The roots from each plot were then scored by comparing them individually with these standards. The scores for each plot were then totaled and divided by the number of roots on that plot. This average value was considered an estimate of the extent of nematode damage. Higher scores indicate greater injury. The plot scores and the yields from each treatment are summarized in tables 1, 2, 3, and 4.

All Fall treatments except the furyl-nitro-ethylene dust gave significant reductions in nematode injury on the spring tomato crop (Table 1 and Fig. 1, B). These reductions were reflected in significantly greater yields. A coefficient of correlation of -0.78 was found between yield and degree of

TABLE 1.—*Effect of treating heavily infested soil with various fumigants on the incidence of root-knot nematode, and the yield and value of a spring tomato crop*

Treatment	Dosage[a]	Ave. root scores	Ave. yield per plant	Ave. weight per fruit	Ave. fruits per plant	Ave. cash income per plant
			Lb.	*Lb.*	*No.*	
Check	0	2.75	4.92	0.35	14.1	$1.15
Furyl-nitro-ethylene dust	2 lb.	2.61	5.03	0.33	15.2	$1.16
Chloropicrin	2 cc.	1.70	6.00	0.36	16.6	$1.36
Ethylene dichloride	15 cc.	1.11	6.23	0.36	17.3	$1.44
Methyl bromide mixture (10%)	5 cc.	0.60	6.00	0.35	17.0	$1.35
Least diff. req. for odds	19:1	0.50	0.64	0.04	1.4	0.21
	99:1	0.70	0.90	0.05	2.0	0.29

[a] With the dust treatment 2 lb. dosage represents amount used per 100 square feet. With the liquids, figures give amount used per injection. Injections 10 inches apart.

nematode injury. Failure of the dust treatment was disappointing, because this material had appeared very effective in small-scale preliminary trials. Possibly, inability to obtain a satisfactory mixing of the dust with the soil was the main cause of its failure under commercial conditions. Use of a mechanical rotary tiller to incorporate it with the soil, however, gave no better results. Better nematode control was obtained with the methyl bromide mixture than with either chloropicrin or ethylene dichloride, but all three produced about the same yields. The relatively ineffective control with chloropicrin may have been due to the fact that treatment was made before nematode galls from the previous crop had thoroughly rotted.

The average cash income per plant was computed by multiplying the yield for each day by the price obtained on that day. Since the nematodes had more effect on the plants in the untreated plots as the season progressed, the greater part of the increase in yield on the treated plots over the check plots occurred near the end of the season, at which time prices were low (Fig. 2). It is obvious, therefore, that multiplying the total yield from each treatment by an average price for the season, as is often done, would have given too high a value to the increased income due to treatment. For

example, if it were assumed that a treated and an untreated plot yielded the same until the last two pickings, at which time the treated plot yielded 5 pounds more than the check, the apparent increase in income would have been $1.25 if the yields for the season were multiplied by the average seasonal price (*i.e.*, 25¢ a pound). However, the actual increase in income, obtained by multiplying by the value of the tomatoes when picked (*i.e.*, 5¢ a pound), would have been only 25¢.

When the price is constant throughout the picking season, as is frequently the case with the fall crop, the use of an average price may be justifiable in computing increased income due to treatments. This was the case in the fall experiment recorded in table 8.

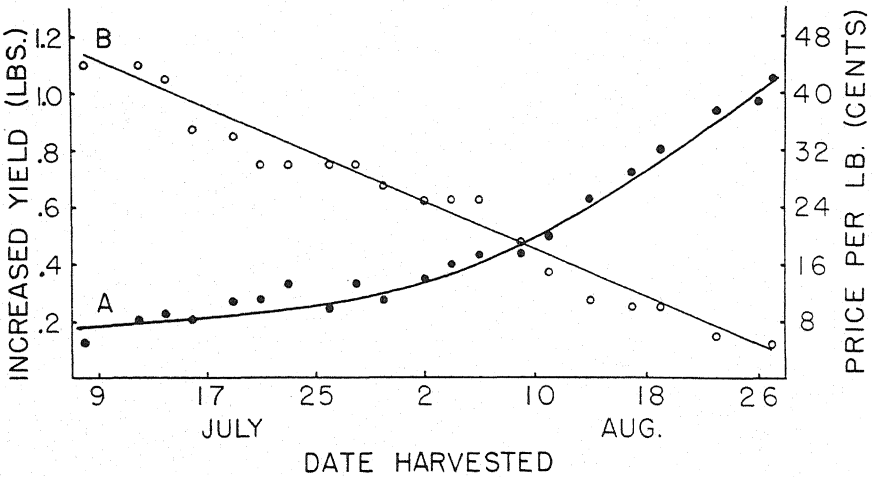


FIG. 2. Cumulative increase in tomato yields (A) (on a per plant basis) of chloropicrin-treated plots over the untreated plots, and price of tomatoes (B) throughout the season. Note that yield (A) is expressed as cumulative or total increase and that greatest increase came at the end of the season when prices were lowest. Ethylene dichloride and the methyl bromide mixture each gave similar curves.

All treatments listed in table 2 significantly reduced nematode injury. However, the nematode damage to the checks was not sufficient to reduce yields to the point where differences could be demonstrated statistically. When one realizes that the yield of the plants in the untreated plots was not markedly reduced, even when the roots were injured to the extent shown in figure 1, C, it is easy to understand why differences in yield between treatments are difficult to demonstrate.

The data in tables 3 and 4 indicate that 3- to 4-cc. injections of the 10 per cent methyl bromide mixture gave sufficient nematode control to permit normal yields of tomatoes. Increasing the dosage to 7 cc. resulted in better nematode control (Fig. 1, D), but yields were not improved. It appears that a low dosage that will reduce the nematode population sufficiently to permit young plants to get a good start will result in satisfactory yields by the current crop. However, if a second high-temperature crop suscep-

TABLE 2.—*Effect of treating lightly infested soil with various fumigants on the incidence of root-knot nematode and the yield and value of a spring tomato crop*

Treatment	Dosage per injection	Ave. root scores	Ave. yield per plant	Ave. weight per fruit	Ave. fruits per plant	Ave. cash income per plant
	Cc.		*Lb.*	*Lb.*	*No.*	
Check	0	2.04	6.87	0.37	18.7	$1.80
Chloropicrin–ethylene dichloride mixture (1–9)	10	0.67	7.23	0.35	20.6	$1.82
Methyl bromide mixture (10%)	5	0.51	7.10	0.36	19.6	$1.79
Chloropicrin	2	0.40	7.38	0.36	20.7	$1.89
Chloropicrin–methyl bromide mixture (3–1)	2	0.14	7.47	0.37	20.1	$1.88
Least diff. req. for odds	19:1	0.46	0.90	.....	2.05	0.18
	99:1	0.64	1.26	.....	2.87	0.25

tible to nematodes is to follow the first, then a more effective dosage might be desirable. The value of a low-dosage treatment before each crop as compared with a heavy treatment for two crops remains to be established. The optimum dosage may well depend on the crops grown, as well as amount of inoculum in the soil, soil conditions, etc.

When treatments resulted in an increase in yield this increase was due to the production of more fruits per plant rather than to an increase in the size of the individual fruits (Tables 1, 2, and 3). The production of fewer fruits per plant on the untreated plots was not due to early death of plants, as these were still green at the close of the picking season.

TREATMENTS FOR FALL TOMATO CROP

Another series of soil treatments for the control of the root-knot nematode was made in a large commercial greenhouse at Schenectady, N. Y.[6] Treatments were made on June 25 in raised beds from which tomato plants,

TABLE 3.—*Effect of dosage of the 10 per cent methyl bromide mixture on the incidence of root-knot nematode, and the yield and value of a spring tomato crop*

Dosage per injection	Ave. root scores	Ave. yield per plant	Ave. weight per fruit	Ave. fruits per plant	Ave. cash income per plant
Cc.		*Lb.*	*Lb.*	*No.*	
0	2.87	5.02	0.37	13.5	$1.18
3	1.80	5.66	0.37	15.6	$1.37
5	0.86	5.69	0.39	14.4	$1.38
7	0.51	5.91	0.39	15.3	$1.37
Least diff. req. for odds					
19:1	1.40	0.90	.....	2.2	0.19
99:1	1.71	1.10	.....	3.3	0.28

[6] Acknowledgment is made to the Brownsey and Marks Greenhouse Company for their cooperation.

TABLE 4.—*Effect of dosage of the 10 per cent methyl bromide mixture on the incidence of root-knot nematode, yield, and value of a spring tomato crop*

Dosage	Ave. root score	Ave. yield per plant	Ave. income per plant
Cc.		*Lb.*	
0	3.65	3.64	$1.00
4	1.69	5.21	$1.21
5	1.75	4.64	$1.34
6	1.31	5.69	$1.22
7	1.48	4.18	$1.43
Least diff. req. for odds			
19:1	0.60	1.09	0.30
99:1	0.87	1.59	0.44

heavily infested with nematodes, had been removed about June 1. The soil was loosened by forking just before treatment. Each of the treatments listed in table 5 was replicated five times in randomized plots. The dosage applied per injection was computed by dividing the total amount of fumigant used on the 5 replicates by the total number of injections made. About the first of August, 14 tomato plants of the variety Vetomold were set in each plot. Records were kept by the grower of the yields from each plot at each picking. At the end of the growing season the authors examined and scored the roots of each plant. The 6 standard roots used for scoring in this experiment are illustrated in figure 3. The results are in table 5.

All treatments gave very satisfactory nematode control. No significant differences in control were obtained between any of them, although the 10 per cent methyl bromide mixture appeared most effective, at the three rates employed, the chloropicrin alone and chloropicrin mixtures came next, and the D–D mixture was the poorest. All treated plots produced more fruit than the untreated, but none of the differences in yield between the treated plots was significant. The low dosage rates of the 10 per cent methyl

TABLE 5.—*Effect of treating nematode-infested soil with various fumigants on the incidence of root-knot nematode and the yield of a fall tomato crop*

Fumigant	Dosage per injection	Ave. root score	Ave. yield per plant
	Cc.		*Lb.*
Check	0.0	2.81	4.54
Methyl bromide mixture (10%)	4.19	0.70	5.65
" " " "	6.16	0.67	5.38
" " " "	8.64	0.76	5.47
Chloropicrin	1.61	0.95	5.50
"	2.58	0.98	5.67
Chloropicrin–ethylene dichloride mixture (1–9)	7.35	0.99	5.35
Chloropicrin–methyl bromide mixture (3–1)	1.80	0.96	5.80
D–D mixture	4.18	1.25	5.45
Least diff. req. for odds 19:1	.....	0.72	0.66
99:1	.....	0.97	0.89



FIG. 3. Roots selected from fall crop as standards by which to score those from the experimental plots. They represent (left to right) classes 5, 4, 3, 2, 1, and 0.

bromide mixture (4.19 cc.) and of the straight chloropicrin (1.61 cc.) resulted in as good yields as treatments at the higher rates of 8.64 and 2.58 cc., respectively. The addition of methyl bromide to chloropicrin appeared more effective than either chloropicrin alone or the chloropicrin–ethylene dichloride mixture. The lower effectiveness of D–D mixture at the 4-cc. rate (6 cc. per square foot) employed was not anticipated in view of results obtained elsewhere with much lower dosages. Possibly this can be attributed to differences in soil type or organic content.

The cost per 1,000 square feet of the various chemicals and mixtures used in these experiments is in table 6. The cost of application is excluded, since this is approximately the same for all of these materials. Differences in total costs of treatments are chiefly due to the prices and quantities of the chemicals used. Application costs may vary from 25¢ to 75¢ per 1,000 square feet, depending on the labor costs, size of area treated, equipment

TABLE 6.—*Cost of the chemicals used for soil fumigation*

Fumigant	Cost per pound	Approx. No. of cc. per pound	Amount per injection on 10-in. centers		Pounds required to treat 1000 sq. ft.		Approx. cost of chemicals to treat 1000 sq. ft.	
			Min.	Max.	Min.	Max.	Min.[b]	Max.[c]
	Cents		*Cc.*	*Cc.*				
D–D mixture	11–15[a]	384	1.0	4.0	3.8	15.0	$0.42	$2.25
Methyl bromide mixture (10%)	16–20	329	3.0	9.0	13.2	39.5	$2.10	$7.90
Ethylene dichloride	8–10	361	10.0	15.0	39.9	59.9	$3.20	$6.00
Chloropicrin–ethylene dichloride mixture (1–9)	16–18	350	7.5	10.0	30.9	41.2	$4.95	$7.40
Chloropicrin–methyl bromide mixture (3–1)	78–83	271	1.5	2.0	8.0	10.6	$6.25	$8.80
Chloropicrin	80–85	274	1.5	2.5	7.9	13.1	$6.30	$11.15
Methyl bromide	70–75	262						

[a] Limited availability. Market price not yet fixed.
[b] Calculated from figures for lowest cost and minimum dosage.
[c] Calculated from figures for highest cost and maximum dosage.

available, etc. Thus, total cost of treatment ranges from about $2.50 to almost $10.00 per 1,000 square feet, which in general is well below the range recorded by Newhall (5) for steaming in commercial greenhouses ($6.50 to $20.00 per 1,000 square feet).

The data in table 7, showing economic value of treatments for greenhouse tomatoes on an individual plant basis, computed from results on the spring crop, and in table 8, computed from the fall experiment, indicate the relatively low cost of treatment in relation to the income from the crop.

TABLE 7.—*Economic value of soil treatments in a tomato greenhouse on a per plant basis. Spring of 1943*

Fumigant	Cash income[a]	Cost to treat	Increased income
		Cents	*Cents*
Check	$1.14	.....	.....
Methyl bromide mixture (10%)	$1.35	1.8	18.2
Chloropicrin	$1.36	3.7	17.6
Ethylene dichloride	$1.44	2.5	26.8

[a] Values taken from table 1.

TABLE 8.—*Economic value of soil treatments in a tomato greenhouse on a per plant basis. Fall of 1943*

Fumigant	Dosage	Cash income[a]	Cost to treat	Increased income
	Cc.		*Cents*	*Cents*
Check	.....	$1.13	.....	.....
Methyl bromide mixture (10%)	4.2	$1.41	1.0	26.8
" " " "	6.2	$1.34	1.5	19.5
" " " "	8.6	$1.37	2.1	21.2
Chloropicrin	1.6	$1.37	2.3	21.7
"	2.6	$1.42	3.8	24.5
Chloropicrin–ethylene dichloride mixture (1–9)	7.4	$1.34	1.6	18.7
Chloropicrin–methyl bromide mixture (3–1)	1.8	$1.45	2.6	28.9
D–D mixture	4.2	$1.36	0.7	22.1

[a] Since the price did not vary materially throughout the picking season on this crop the income was computed by multiplying the yield (Table 5) by the average price for the season (25 cents a pound).

Effectiveness of treatment should be the primary consideration with high value crops, particularly in periods of high prices. While it is realized that these income figures may be somewhat high, nevertheless, even in normal times, the value of most greenhouse crops is sufficiently high to require only slight increase in yield to pay for the cost of treatment. For low value, or outdoor crops, the cost of treatment may become the determining factor in selecting a treatment.

DISCUSSION

The ideal soil fumigant for treatment of greenhouse soils should possess certain characteristics. It should be effective against all types of soil-borne

pathogens and weed seeds. The fumes should not be toxic, irritating or uncomfortable to work in. The vapor escaping from the soil should not be so phytocidal as to prohibit treating areas adjacent to growing plants. The fumigant should be able to successfully penetrate heavy soils and unrotted roots therein. The vapor should escape rapidly enough from the soil or be sufficiently nontoxic to growing plants to permit planting a few days after treatment. Materials should be readily available, low-priced, and noncorrosive to metals.

No one fumigant can be said to be the best in all instances, because the relative importance of the characteristics just enumerated varies under different circumstances. Chloropicrin appears more effective against a wider range of soil pests and weed seeds than any of the fumigants tested. Methyl bromide has some fungicidal and herbicidal value, though probably less than chloropicrin at the concentrations recommended for nematode control. Ethylene dichloride appears to have relatively little value as a fungicide or herbicide. The merits of D–D mixture in these respects remain to be established. It appears, therefore, that if the control of certain fungi or weeds is important, chloropicrin treatment would probably be the most satisfactory.

If nematode control is the primary purpose of soil fumigation, other fumigants have certain advantages over chloropicrin. If a grower wants to replant as soon as possible, methyl bromide mixture is more satisfactory, since it appears to penetrate unrotted roots, and it escapes from the soil quickly so that replanting can take place a few days after treatment. The efficacy of chloropicrin may be reduced if root galls from the previous crop are not given sufficient time to decay before the treatment is made. No information on the penetrating power of ethylene dichloride or D–D mixture into unrotted roots has been obtained. Ethylene dichloride dissipates from the soil fairly rapidly. Toxic quantities of D–D mixture appear to persist in the soil for even longer periods than will chloropicrin. Soil conditions greatly influence the length of retention of any of these chemicals, but their rate of dissipation seems to be in the order indicated.

The lachrymatory effects of chloropicrin make it disagreeable, but it is safe to work with. Methyl bromide mixtures are not disagreeable to handle, but may be somewhat dangerous because of their toxicity to humans. If proper precautions are observed, harmful effects appear unlikely. Neither ethylene dichloride nor the D–D mixture are seriously toxic or especially disagreeable, but breathing vapor of D–D mixture for long periods may have a nauseating effect, and both chloropicrin and D–D may cause chemical burns if left in contact with the skin.

Chloropicrin vapor is very toxic to foliage, which necessitates removing all plants from a house before it may be safely treated. Plants appear more tolerant to the other fumigants, which means that under certain circumstances one area in a greenhouse may be treated without removing plants from nearby areas.

Cost of treatment is not an extremely important factor in selecting a material if high-value crops are to be grown, but the low costs of D–D and the methyl bromide mixture permit their use for low-value crops.

Neither the methyl bromide mixture nor ethylene dichloride have a marked corrosive effect on metals. Consequently, these chemicals can be applied with certain applicators which would be unusable with more corrosive materials, as chloropicrin and D–D mixture.

The advantage of using mixtures of fumigants is often questionable. Adding methyl bromide to chloropicrin results in a mixture which has good penetrating power and is fungicidal, but the mixture is more difficult to handle than chloropicrin alone and the lachrymating and corrosive features of chloropicrin are still present. Mixing ethylene dichloride with chloropicrin results in a more economical fumigant than chloropicrin, but it is still disagreeable to use and the effectiveness seems to be proportional to the amount of chloropicrin. On the other hand, mixing methyl bromide with other chemicals is necessary to keep it from vaporizing too rapidly at ordinary temperatures. Methyl bromide mixtures other than the ones used in these experiments may prove more valuable as soil fumigants.

The chief advantages of soil fumigation with chemicals over heat treatment, such as steam or hot water, lie in their greater economy and lower equipment and labor requirements. Steam treatment, however, is more effective against a wider range of soil pests, can more safely be made in houses containing growing plants, and usually permits the soil to be replanted more promptly.

Hand injectors were employed in these experiments: a "Larvajector,"[7] model RR for chloropicrin, model A for D–D and a "Mack's Chemical Injector"[8] for other fumigants. In more recent tests, still under progress, a two-row, continuous-flow, power-driven type of applicator has been used in comparison with hand-operated injectors. Use of such apparatus reduces labor and time, thus adding to the practicability of fumigation as a method of controlling soil-borne pests.

SUMMARY

The efficacies of several soil fumigants against *Heterodera marioni* were compared in two commercial tomato greenhouses. Control of root knot was measured by comparing yields and by scoring all roots on several series of replicated plots.

The following materials, listed alphabetically, and rates of application gave good control when injected 4–5 inches deep on 10-inch centers:

Chloropicrin (Larvacide), used at 1.6, 2, and 2.5 cc. per injection.

Chloropicrin plus ethylene dichloride (1 to 9) at 7.35 and 10 cc. per injection.

[7] Procured from Innis, Speiden and Co., Niagara Falls, N. Y. Delivers up to 4 or 5 cc. per injection.

[8] Procured from Stauffer Chemical Co., New York. Delivers up to 60 cc. per injection.

Chloropicrin plus methyl bromide (3 to 1) at 1.8 and 2.0 cc. per injection.

D–D mixture, containing approximately equal parts of 1,2-dichloropropane and 1,3-dichloropropylene, used at 4 cc. per injection.

Ethylene dichloride at 15 cc.

Methyl bromide mixture (Dowfume Br. 10), containing 10 per cent methyl bromide in a 3 to 1 mixture of ethylene dichloride and carbon tetrachloride, used at 3, 4, 5, 6, 7, and 8 cc. per injection.

The advantages and disadvantages of each are discussed. Furyl-nitroethylene dust at 20 pounds per thousand square feet failed to control root knot.

Increasing the dosage of chloropicrin above 1.6 cc. and of the 10 per cent methyl bromide mixture above 3 cc. per injection did not result in larger yields, even though more complete control of nematodes was obtained.

Increased yields from the treated plots were due to the production of more tomato fruits, rather than to an increase in their average size.

Certain of the materials have advantages over chloropicrin for nematode control either because they are cheaper, easier to handle, or permit replanting sooner. The 10 per cent methyl bromide mixture appears very promising in all these respects.

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THE USE OF FUNGICIDES AND GROWTH SUBSTANCES IN THE CONTROL OF FUSARIUM SCALE ROT OF LILIES

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Easter lilies (*Lilium longiflorum* Thunb.) are propagated from bulb offsets, stem bulblets, and scales. These last are fleshy overlapping segments of the bulb and afford the most rapid means of increasing desired types. However, when the scales are removed from the bulbs their injured bases are vulnerable to attack by the basal rot organism, *Fusarium oxysporum* f. *lilii* Imle. Some of the new Easter lily clons developed at the Plant Industry Station, Beltsville, Maryland, are very susceptible to this rot. Imle (9) states that the culture of many other lily species, such as *L. auratum* Lindl., *L. Browni* Poit., *L. bulbiferum* L., *L. candidum* L., *L. formosanum* Stapf, *L. speciosum* Thunb., *L. testaceum* Lindl., and *L. Willmottiae* Wils., is limited by this disease.

In an effort to find a suitable fungicide which could be applied to scales for protection against rot, a number of materials and combinations of materials have been tested on scales of *Lilium longiflorum* and *L. testaceum*. Certain of these fungicides controlled Fusarium rot and in addition some of them stimulated root and shoot growth when used alone or as carriers for synthetic growth substances.

MATERIALS

Lilium longiflorum and *L. testaceum* bulbs used in these tests were grown at the Plant Industry Station, Beltsville, Maryland, in 1943. After harvest the bulbs were packed in moist peat and held in common storage until used.

The method of handling the scales in greenhouse tests has been described in detail elsewhere (10, 14). Essentially it consists in removing the scales from the bulbs, treating them with growth substances, placing them on sand on greenhouse benches, and covering with 2 to 3 inches of wet sphagnum moss. Bulblets and roots form in from 3 to 6 weeks, and the scales may then be planted in flats or in the field.

The following materials were employed in the scale treatments: Fermate (ferric dimethyldithiocarbamate, Du Pont Semesan Co.); Zincate (zinc dimethyldithiocarbamate, R. T. Vanderbilt Co.); Spergon (98 per cent tetrachloro-para-benzoquinone plus 1 per cent of a buffering agent, U. S. Rubber Co.); New Improved Ceresan[3] (5 per cent ethyl mercury phosphate, Du Pont Semesan Co.); Arasan (50 per cent tetramethyl thiuramdisulfide, Du Pont Semesan Co.); 37 to 40 per cent formalin solution; naphthaleneacetic acid; and indolebutyric acid.

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[3] Hereafter in this paper New Improved Ceresan will be referred to as Ceresan.

METHODS AND RESULTS

Experiment 1. Scales from bulbs of a clon, No. 224–34, which had been very susceptible to *Fusarium* in the field, were treated as follows: Some were dipped momentarily in a suspension of Fermate or of Zincate, each at the rate of 1 pound to 100 gallons of water; others were dipped for 2 minutes in a suspension of Ceresan at the rate of 1 pound to 40 gallons of water; a fourth lot was coated with Arasan at the rate of 2 ounces to 60 pounds of scales; a fifth lot was coated with Spergon at the rate of 2 ounces per 100 pounds of scales; and a sixth lot was soaked for 30 minutes in a 2 per cent formaldehyde solution (1:50 dilution of commercial formalin). The Spergon and Arasan were applied by shaking the weighed scales in a paper sack containing the required amount of material. Approximately 150 scales were treated with each fungicide, then separated into 3 lots, placed in 3 different plots on a greenhouse bench, and covered with sphagnum.

Thirty-three days later the scales were examined and photographed. Those treated with Spergon or Arasan were protected from scale rot and had by far the greatest development of roots, bulblets, and shoots (Fig. 1). Ceresan afforded excellent protection from rot but inhibited bulblet formation. Scales treated with formaldehyde showed considerable surface injury. Fermate and Zincate were relatively ineffective as protectants.[4]

Experiment 2. Growth stimulation resulting from Arasan and Spergon in Experiment 1 suggested that if these materials were combined with Ceresan the inhibiting effect of the latter might be overcome. It also seemed desirable to test combinations of each fungicide with a number of root-inducing chemicals that have been recommended as aids in propagation (10). The latter furnish no protection against rot and may cause injury to the scales.

A second experiment was set up in which the treatments included combinations of fungicides and growth substances. Two types of scales were used. The first type was from a mixture of Easter lily clons that were apparently free of Fusarium rot when dug and scaled. The second type was from a Fusarium-susceptible clon, No. 38. No scales showing evidence of disease were used. The following treatments were applied to scales of each type: Ceresan, Spergon, Arasan, naphthaleneacetic acid 1:5000 in talc, indolebutyric acid 1:5000 in talc, 2 per cent formaldehyde, a 30-minute water soak, and an untreated control. The following combinations were also used: Ceresan and Spergon, Ceresan and Arasan, Spergon and naphthaleneacetic acid, Spergon and indolebutyric acid, Arasan and naphthaleneacetic acid, Arasan and indolebutyric acid, 2 per cent formaldehyde solution containing 0.001 per cent naphthaleneacetic acid, and 2 per cent formaldehyde solution containing 0.001 per cent indolebutyric acid. Scales were treated with Ceresan as in Experiment 1. When Arasan or Spergon were used either alone or as carriers for the growth substances, weighed amounts of the powders were not used but the scales were coated by shaking them in

[4] In more recent tests Fermate dusted on lily scales has provided excellent protection against rot.

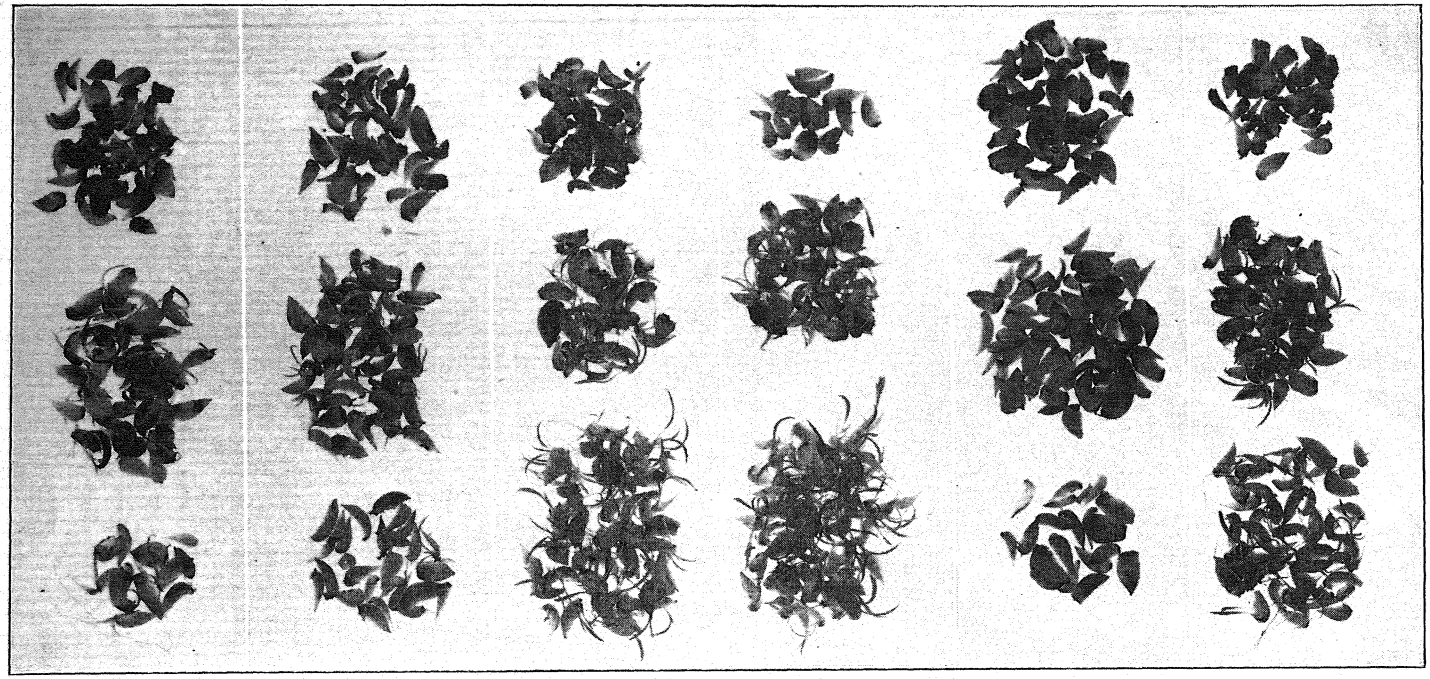


FIG. 1. Fusarium-susceptible Easter lily scales 33 days after treatment with the following materials: A, Fermate; B, Zincate; C, Spergon; D, Arasan; E, New Improved Ceresan; and F, formaldehyde. Upper row (all treatments), infected scales without bulblets; middle row (with the exception of treatment E), infected scales with bulblets; and lower row, healthy scales, with and without bulblets. Middle group of treatment E, healthy scales without bulblets; and lower group of E, healthy scales with bulblets. There were approximately 150 scales in each treatment. Those scales that were entirely rotted are not included.

a paper bag with an excess of these materials. When Ceresan was used with Spergon or with Arasan the scales were first dipped for 2 minutes in a suspension of 1 pound of the mercurial to 40 gallons of water, allowed to dry, then dusted with Arasan or Spergon. In all other treatments in which combinations were used the materials were combined prior to application. Scales were soaked for 30 minutes in the formaldehyde solutions.

The naphthaleneacetic and indolebutyric acid mixtures were prepared at a concentration of 1 part in 5000 parts of talc, Spergon, or Arasan. The acids were dissolved in sufficient 95 per cent ethyl alcohol to wet thoroughly

TABLE 1.—*Numbers of healthy scales, scales with bulblets, and bulblets on two groups of Easter lily scales 40 days after treatment with fungicides and growth substances. One hundred and fifty scales from each group were used for each treatment*

Treatment	Mixture of clons[a]			Clon No. 38		
	Healthy scales	Scales with bulblets	Bulblets	Healthy scales	Scales with bulblets	Bulblets
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
New Improved Ceresan	122	110	154	84	62	85
Spergon	109	144	242	67	111	168
Arasan	129	148	258	103	124	184
Naphthaleneacetic acid 1:5000	26	119	197	32	95	140
Indolebutyric acid 1:5000	23	109	165	19	59	92
New Improved Ceresan + Spergon	129	94	153	96	65	86
New Improved Ceresan + Arasan	140	142	287	125	126	207
Spergon + naphthaleneacetic acid 1:5000	110	145	266	61	116	189
Spergon + indolebutyric acid 1:5000	111	144	267	68	101	138
Arasan + naphthaleneacetic acid 1:5000	127	149	276	116	129	227
Arasan + indolebutyric acid 1:5000	134	145	268	120	130	212
2% formaldehyde + 0.001% naphthaleneacetic acid[b]	17	130	223	3	71	106
2% formaldehyde + 0.001% indolebutyric acid[b]	3	118	210	0	77	115
2% formaldehyde[b]	5	124	217	9	79	111
Untreated, dry	51	134	199	22	60	74
Untreated, water 30 minutes	32	130	215	17	66	82
Least difference necessary for significance between treatment totals with odds of						
19:1	19.6	12.5	36.9	20.6	25.7	35.5
99:1	26.4	16.8	49.7	27.8	34.6	47.4

[a] Bulbs from these clons appeared to be healthy when scaled.
[b] Scales were injured by the formaldehyde treatment.

the talc or fungicide used as a carrier. After the alcohol had evaporated the mixtures were broken up and stored in darkness.

In each treatment there were 150 scales from clon No. 38 and 150 from the mixed clons. They were divided into lots of 50 each and placed at 3 locations in a greenhouse bench. All scales were covered with sphagnum that was kept moist. The temperature in the greenhouse was 65° to 70° F. during the day and 60° to 65° at night.

The scales were treated October 21, 1943, and examined November 30,

1943. The scales from each treatment were sorted into 2 groups depending upon the presence or absence of bulblets. Each group was further subdivided according to presence or absence of Fusarium infection of the scales. The healthy scales, scales healthy but without bulblets, total scales with bulblets, and total bulblets were counted. These data, except for the number of scales healthy but without bulblets, are in table 1. Scales entirely rotted are not included in the tabulation. It is quite possible that healthy bulblets could be produced from many of the scales not classed as healthy, since lesions frequently were present only at the tips or edges of scales farthest from bulblet production areas. *Fusarium* was the only organism obtained from numerous isolations made from the decaying areas of these scales.

All materials and combinations except naphthaleneacetic acid, indolebutyric acid, and formaldehyde afforded very good protection from *Fusarium*. Of the materials tested individually, Arasan provided the best protection; combined with Ceresan it was even more effective. Arasan and all the combinations in which it was used provided much better protection than did Spergon alone or in the same combinations. The differences between Arasan and Spergon were much greater when used on the Fusarium-susceptible clon No. 38 than when used on the less susceptible mixture of clons. Scales treated with indolebutyric acid or naphthaleneacetic acid did not appear to be injured, but those treated with formaldehyde, alone or in combination with either of these 2 growth substances, were injured. This formaldehyde treatment has been recommended for use on lily bulbs for the control of Fusarium basal rot (9), but it appears to be too severe for use on lily scales.

As in the first experiment, Ceresan inhibited bulblet formation. Seventy of the 300 scales treated with Ceresan in Experiment 2 had not developed bulblets at the time records were taken. In a similar lot of scales treated with Ceresan followed by Arasan only 19 lacked bulblets. On the other hand there were 92 scales without bulblets when Spergon followed the Ceresan treatment. In the other treatments, except the Spergon-indolebutyric acid combination, there were very few (0 to 16) scales without bulblets. Some of the scales from the mixed clons given the Ceresan, Arasan, or Ceresan-Arasan treatments as well as some of the scales not treated were removed from the sphagnum and planted in flats of soil in the greenhouse December 6, 1943. On February 8, 1944, the scales were removed from the soil and photographed (Fig. 2).

Whenever Arasan or Spergon was used, with the exception of the Spergon-Ceresan combination, there were significantly more bulblets than on untreated scales. Arasan treatments yielded more bulblets than the Spergon treatments. Naphthaleneacetic acid in talc applied to scales of clon No. 38 was the only other treatment that resulted in the production of significantly more bulblets than the untreated checks. The greatest root and shoot development and bulblet production occurred when Arasan was used as the carrier for naphthaleneacetic acid (Fig. 3). In general, at the concentra-

tions used, naphthaleneacetic acid resulted in the production of more bulblets than did indolebutyric acid.

Experiment 3. Bulbs of *Lilium testaceum* badly infected with *Fusarium* were scaled and treated with Arasan on November 20. Before treatment the



FIG. 2. Easter lily scales from a mixture of clons 110 days after treatment with New Improved Ceresan (upper row), New Improved Ceresan followed by Arasan (second row), Arasan (third row), and untreated (lower row). Arasan in combination has overcome the inhibiting effects of the Ceresan. Note the rot in the untreated scales.

most severely rotted scales were discarded and the rotted areas of the others removed with a knife. When the scales were examined December 18, 176 of the 189 treated scales (93 per cent) showed no sign of rot whereas only 17 of the 62 untreated ones (27 per cent) were free of decaying areas. There were

274 bulblets (1.4 per scale) on the treated scales compared with but 35 (0.6 per scale) on those untreated.

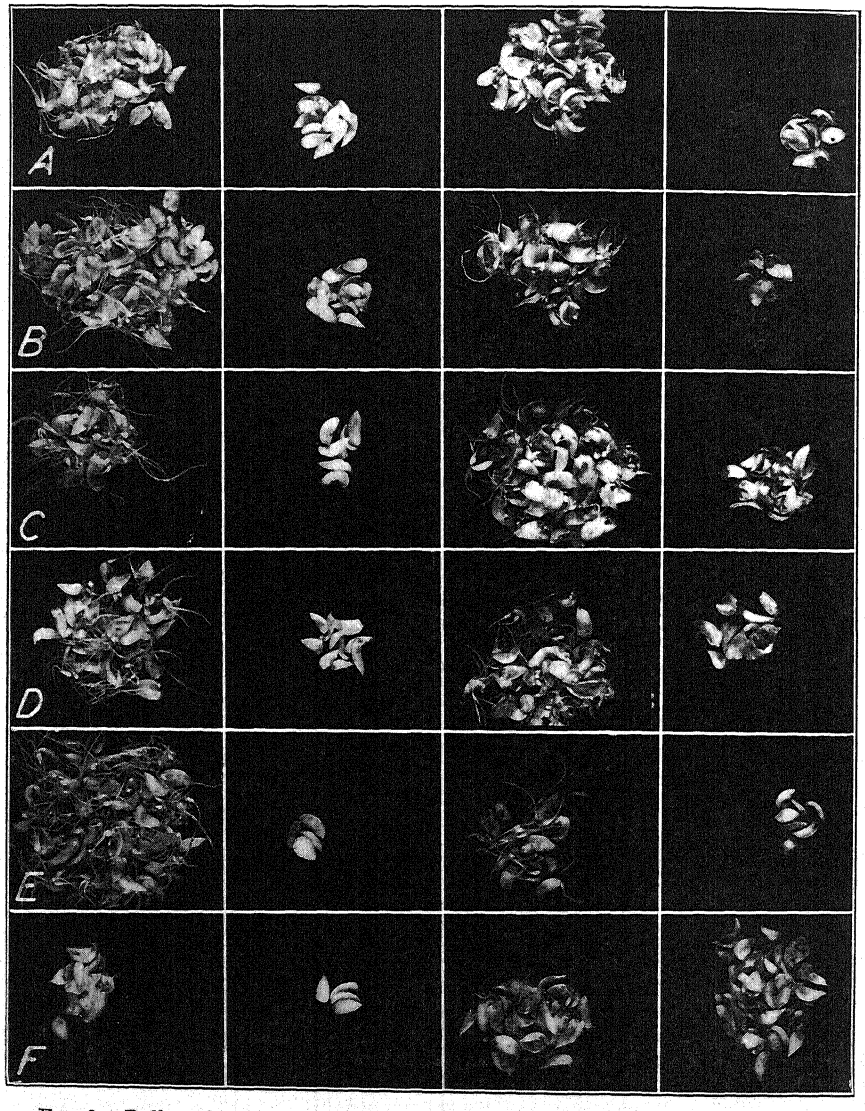


FIG. 3. Bulb scales from a mixture of Easter lily clons 40 days after the following treatments: A, Spergon alone; B, Arasan alone; C, naphthaleneacetic acid alone; D, Spergon as the carrier for naphthaleneacetic acid; E, Arasan as the carrier for naphthaleneacetic acid; and F, untreated checks. One hundred and fifty scales were used in each treatment. From left to right the groups are: healthy scales with bulblets, healthy scales without bulblets, diseased scales with bulblets, and diseased scales without bulblets. Those scales that were entirely rotted are not included.

DISCUSSION

Mercurials and other fungicides have been widely tested as seed protectants. Increased yields resulting from such treatments have been as-

cribed for the most part to better protection of the treated seed during pre- and post-emergence. However, McNew reported evidence for stimulation of pea growth following treatment with Spergon (11).

In the present study Spergon was very effective in protecting Easter lily scales from Fusarium rot during propagation. It was inferior, however, to Arasan in protective action when scales from a very susceptible clon were used. Both fungicides exerted a marked growth-stimulating effect on the treated scales. Part of this stimulation undoubtedly arose from the fact that the Fusarium infection was reduced, but there appears to be more growth acceleration than could be accounted for by this action alone. It has been suggested that the stimulation from the Arasan occurs because of its nitrogen content. This possibility remains to be established. The growth-stimulating properties of Spergon must be explained in some other manner since it contains no nitrogen.

Ceresan afforded good protection against *Fusarium* on lily scales. Its use inhibited bulblet and root formation. This inhibition was largely overcome by treatment with Arasan, thus offering further evidence of the growth stimulating properties of the latter compound. Spergon was ineffective in overcoming Ceresan inhibition. Growth inhibition of lily bulbs following treatment with mercurials has been reported (13).

Growth substances and fungicides recently have been combined for use in seed treatment tests with conflicting results (1, 2, 3, 12, 15). Grace (5, 6) and Grace and Farrar (8), who used ethyl mercury phosphate combined in talc dusts with indoleacetic and naphthaleneacetic acids on woody and herbaceous cuttings, report occasional beneficial results from the combinations. Ceresan (ethyl mercury phosphate) was not combined in the present study with either naphthaleneacetic acid or indolebutyric acid. However, when these 2 growth substances were combined with either Spergon or Arasan, bulblet formation was increased. The effect of the growth substances was expressed in much heavier rooting of the treated scales. The temporary reduction in growth of the bulblets and leaves which is often associated with heavy root production was eliminated by Arasan or Spergon. Addition of naphthaleneacetic acid or indolebutyric acid to formaldehyde did not result in greater bulblet production nor did their addition reduce the injurious effect of the formaldehyde as has been reported for wheat seed by Grace (4, 7). He added naphthaleneacetic acid and indoleacetic acid to formaldehyde or treated the seeds with the growth substances some time after the formaldehyde treatment and was able by either method to overcome the injurious effect of the formaldehyde.

No evidence of incompatibility between growth substances and fungicides has been reported previously or found in the present studies. The effect of other concentrations of growth substances in combination with these and other fungicides needs to be investigated. Such combinations may prove superior to existing preparations for the propagation of many plant materials owing to the provision for disease protection and growth stimulation

over long periods of time. Further investigations of this problem are in progress.

SUMMARY

Bulb scales of two *Lilium longiflorum* Thunb. clons and scales of *L. testaceum* Lindl., all known to be very susceptible to rot caused by *Fusarium oxysporum* f. *lilii* Imle, and scales of a mixture of less susceptible Easter lily clons were treated with some or all of the following fungicides: Ceresan, Arasan, Fermate, Zincate, Spergon, and formaldehyde. The scales were examined after being planted for 4 to 6 weeks on a greenhouse bench under moist sphagnum. Very good protection was obtained with Arasan and Spergon. Arasan was superior to Spergon on the very susceptible scales. In addition, these 2 materials stimulated production of roots, bulblets, and shoots. Fermate and Zincate were relatively ineffective, and formaldehyde injured the scales. Good protection was obtained with Ceresan but bulblet production was inhibited. When Arasan followed the Ceresan treatment this inhibition was overcome. Spergon did not have this effect.

Arasan and Spergon were used as carriers for the growth substances, indolebutyric acid and naphthaleneacetic acid, at 1 part of growth substance in 5000 parts of fungicide. These combinations produced heavier rooting and more bulblets than did the growth substances or fungicides when used alone. Under the conditions of these tests Arasan was superior to Spergon and naphthaleneacetic acid was better than indolebutyric acid.

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PHYTOPATHOLOGICAL NOTES

Foliage Infection of Lycopersicon esculentum by Colletotrichum phomoides.—The tomato anthracnose fungus, *Colletotrichum phomoides* (Sacc.) Chester, generally is believed to be pathogenic only on mature, or nearly mature, tomato fruits; although at least two workers have reported leaflet and stem infection. According to Rolfs[1] "Anthracnose appears as small brown spots on the stems and leaves . . . these spots enlarge and the greater portion of the leaves may become invaded." Giddings[2] reported, "Stems may be attacked and in some cases the sunken spots may result in considerable damage." These reports were not supported by experimental evidence and apparently have not been generally accepted. The data reported herein demonstrate that under certain conditions *C. phomoides* may infect tomato foliage.

Seedling tomato plants of the varieties Rutgers, Marglobe, Early Baltimore, John Baer, Chicago, and Garden State were grown until 3 to 5 inches tall. Suspensions of spores, sclerotia-like bodies, and mycelium obtained from agar cultures of *Colletotrichum phomoides* were atomized on the plants. Inoculated plants were held in a moist chamber for 1½ to 3 days and then removed to the greenhouse. From 5 to 7 days after inoculation small, necrotic spots appeared on the inoculated leaflets and cotyledons. Uninoculated plants held under the same conditions remained healthy. Close examination, with high magnification, of the lower surface of the inoculated leaflets revealed many small, sunken areas where the mesophyll had collapsed. The central portion later became necrotic, ultimately enlarging to a maximum diameter of 2 mm., and was surrounded by a halo of chlorotic tissue (Fig. 1). Frequently the tips of immature leaflets were attacked, and in such cases the necrotic tissue was more extensive. Lesions on cotyledons were larger than on leaflets and were predominantly at the tip or near the margin. From one-fourth to one-third of the cotyledon was invaded before it abscissed.

Spores typical of *Colletotrichum phomoides* were observed when individual lesions were crushed and examined microscopically. In most instances only 2 to 3 spores were observed for each lesion examined, but in a few cases spores were abundant. Definite acervuli were not seen.

In one experiment at the Riverton laboratory 240 plants were inoculated. All became infected and the number of lesions varied from 4 to 198 per plant. Isolations were made from 30 leaflets 8 days after inoculation. The infected tissue was immersed in a 1–1000 solution of mercuric chloride for 15 minutes and washed in several changes of sterile water to remove surface contaminants. Platings were made on potato dextrose agar. *Colletotrichum phomoides* was recovered from 28 platings, while 2 remained sterile. Two

[1] Rolfs, F. M. Report of the horticulturist. Anthracnose. Florida Agr. Exp. Sta. Ann. Rept. 1905: 45–46. 1906.

[2] Giddings, N. G. Potato and tomato diseases. West Virginia Agr. Exp. Sta. Bul. 165: 19, 20. 1917.

of the recovered isolates were used to inoculate tomato seedlings and in both cases leaflet lesions developed similar to those described. In another isolation experiment 14 infected cotyledons, 20 infected leaflets with necrotic spots, and 20 leaflets with infected tips were surface sterilized and plated on potato dextrose agar. Twelve of the lesions from cotyledons, 18 of the leaflet spots, and 18 of the infected leaflet tips yielded *C. phomoides,* while the

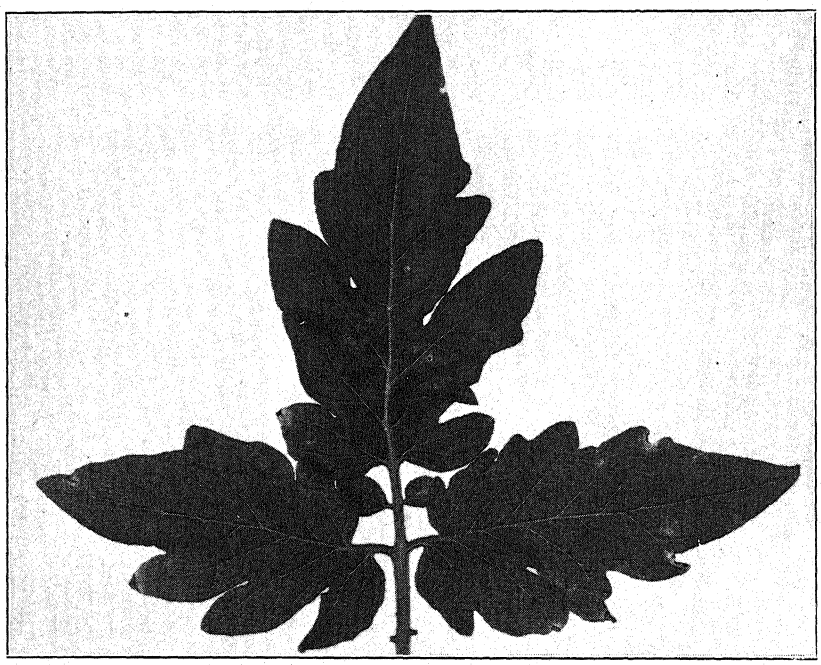


FIG. 1. Tomato leaflets of the Rutgers variety infected with *Colletotrichum phomoides.* Photographed 9 days after inoculation. Small, circular, necrotic spots are surrounded by halo of chlorotic tissue. Marginal infections are larger, irregular, necrotic spots.

remaining 6 platings were sterile.—S. G. YOUNKIN, Research Department, Campbell Soup Co., Riverton, N. J., and A. W. DIMOCK, Cornell University, Ithaca, N. Y.

Intercellular Mycelium of Taphrina deformans in Peach Fruit.—A recent paper by Hildebrand[1] regarding the occurrence of *Taphrina deformans* (Berk.) Tul. on peach fruits has emphasized the paucity of direct evidence for the existence of the organism in such material. Hildebrand was unable to find any trace of the fungus in the maturing fruits that he examined, and, as far as the writers are aware, only three statements[2,3,4] of the actual pres-

[1] Hildebrand, E. M. Mature peach fruits affected by leaf curl. Phytopath. 34: 345–347. 1944.

[2] Clinton, G. P. Report of the botanist for 1913. Conn. Agr. Exp. Sta. Ann. Rept. 1914, Pt. I: 1–42. 1914.

[3] Arnaud, Gabriel, and Madeleine Arnaud. Traité de pathologie végétale. Tome I, Vol. 2, 995–1831. Paris, 1931.

[4] Rose, Dean H., D. F. Fisher, Charles Brooks and C. O. Bratley. Market diseases of fruits and vegetables: peaches, plums, cherries and other stone fruits. U. S. Dept. Agr. Misc. Publ. 228: 26 pp. 1937.

ence of the fungus exist in the many descriptions in the literature of peach fruits which, by their symptomatology and association, were believed to be infected with the leaf curl fungus. The evidence for the existence of *T. deformans* on the fruit, therefore, appears to be chiefly circumstantial. The possibility exists, however, that other factors such as viruses or insects may be involved in the expression of the previously described fruit symptoms—discolored, slightly raised lesions or warty protuberances. In this connection, a comparison of Blodgett's findings on peach wart[5] with some of the descriptions and illustrations of peach fruit presumed to be infected with *T. deformans* reveals a marked similarity in symptomatology between the two diseases. For this reason, examination for the presence of internal mycelium was made of various California collections of half-grown and nearly mature peach fruits with characteristic lesions or warty protuberances, or, in some cases, symptoms intermediate between these two types.

Fruits with discolored, slightly raised lesions similar to those illustrated by Smith[6] were sent to us from Davis, California, by Dr. E. E. Wilson in 1942. Freehand sections revealed a small amount of sub-epidermal mycelium, essentially similar in appearance to that found in infected leaves. In a personal communication, Dr. Wilson stated that he had actually found mature asci and ascospores on the surfaces of some of these peach fruits, an observation similar to those made by Clinton[2] and G. and M. Arnaud[3] on peaches and to that of Cunningham[7] on infected nectarine fruits. Preserved herbarium specimens from unknown localities in the State exhibiting, for the most part, slightly raised lesions were examined microscopically and yielded abundant intercellular, septate mycelium (Fig. 1, A, B, C, D) which was sometimes $2\frac{1}{2}$ mm. below the epidermis. In addition, sub-cuticular mycelium (Fig. 1, E) and ascogenous cells were found. As noted by other investigators,[1,7] the trichomes were scarce or lacking on the surfaces of the slightly raised lesions.

No trace of the fungus was found through histological examination of fresh and preserved specimens from other California collections, most of which consisted of fruits with the warty type of symptom similar to that illustrated by Rose[4] and in which there appeared to be no reduction in the number of trichomes on the surface. Because of the readiness with which the mycelium was demonstrated in fruits with the other type of lesion, it appears doubtful whether these warty specimens were actually infected with the peach leaf curl fungus.

Since some of the fruits examined possessed symptoms that were intermediate between the warty type and the type with slightly raised lesions, it appears that no definite correlation is possible between macroscopic appearance of the fruit and the presence of the fungus. Thus, because of the possibility that factors other than the organism may be responsible for the effects

[5] Blodgett, Earle C. Peach wart. Phytopath. 33: 21–32. 1943.
[6] Smith, Ralph E. Diseases of fruits and nuts. Calif. Agr. Ext. Circ. 120. 1941.
[7] Cunningham, G. H. Leaf curl, bladder plum and cherry curl. Their appearance, cause and control. New Zealand Jour. Agr. 26: 85–97. 1923.

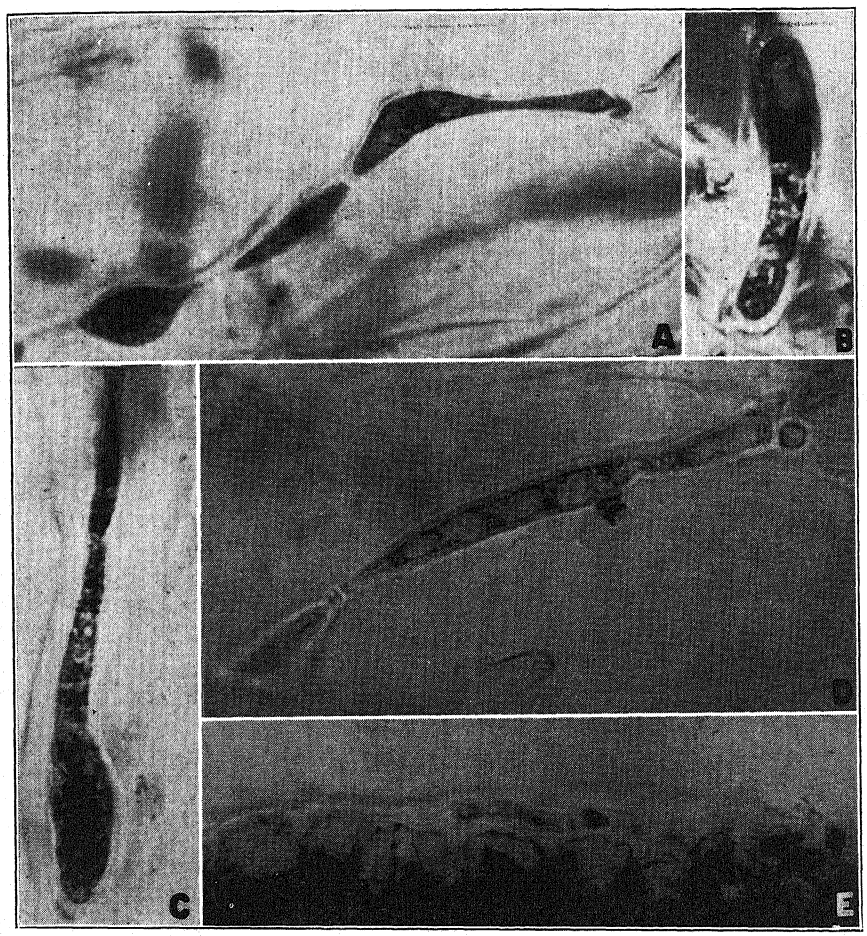


FIG. 1. Intercellular mycelium of *Taphrina deformans* in peach fruit. A, B, C, D. Sub-epidermal hyphal strands. E. Sub-cuticular hyphal strand. Stained with 0.1 per cent cotton blue in lactophenol. (A, D, E, × 850; B, C, × 1200).

observed, it is, in the writers' opinion, unwise to state with assurance that peach fruit is infected with *T. deformans* merely upon the basis of symptomatology.—CATHERINE ROBERTS and J. T. BARRETT, Division of Plant Pathology, University of California, Berkeley 4, California.

Hot-water Treatment for Control of Phytophthora Root Rot of Calla.—Root rot of calla (*Zantedeschia* sp.), caused by *Phytophthora richardiae* Buis., is the most common and destructive disease of this popular ornamental in eastern greenhouses. The damage results from extensive rotting of the roots which leads, in turn, to dying of the foliage and partial or complete failure of the flower crop. In most cases the pathogen penetrates very slightly into the rhizome tissue at the point of attachment of the roots and remains viable in such lesions for many months under the usual conditions

of handling. Such infected rhizomes thus serve to carry the pathogen from one season to the next within an individual greenhouse, and serve as the principal, if not the sole, means of disseminating the organism over long distances.

Fortunately, almost complete eradication of the pathogen can be effected by soaking infected rhizomes for 1 hour in a 1–50 formaldehyde solution,[1] in a 1–1000 mercuric chloride solution,[2] or in a suspension of 1 lb. of New Improved Ceresan plus 2 oz. Dreft in 50 gallons of water.[3] The treated rhizomes should be planted in soil and containers free of the pathogen.

The successful use of hot-water treatments in the control of other diseases caused by Pythiaceae (reviewed by Baker and Cummings[4]) suggested that such treatments might here be used in lieu of the chemical treatments for calla rhizome disinfection.



FIG. 1. Control of Phytophthora root rot of calla by hot-water treatment. Left, untreated checks; right, rhizomes soaked for 1 hour at 122° F. prior to planting. Both lots planted in sterilized soil. Photographed after 128 days.

Thirty dormant white calla rhizomes known to have been infected during the 1943 season were lined out on the laboratory table according to size. Every second rhizome was taken for treatment and the remainder were used as untreated controls. Because of the limited sample only one treatment was employed, a hot-water soak at 122° F. (50° C.) for one hour. On the basis of previous work[4] this was thought adequate to kill the fungus, and had been found in extensive trials[5] to be safe for dormant calla rhizomes. The treatment was made November 8, 1943, and both treated and untreated lots were planted in sterilized soil in sterilized pots 2 days later. The two lots were separated by a considerable distance, but were kept in the same greenhouse section. The pots were not in contact with the bench soil.

[1] Buisman, C. J. Root rots caused by Phycomycetes. Meded. Phytopath. Lab. "Willie Commelin Scholten" Baarn. 11: 1–51. 1927.

[2] Tilford, P. E. Calla lily root rot and its control. Ohio Agr. Exp. Sta. Bimonthly Bull. 157: 138–140. 1932.

[3] Creager, D. B. Successful culture of callas requires prevention of diseases. Flor. Rev. 92 (2382): 11–12. 1943.

[4] Baker, K. F., and K. Cummings. Control of Pythium root rot of *Aloe variegata* by hot-water treatment. Phytopath. 33: 736–738. 1943.

[5] Unpublished tests by Stephen Wilhelm, University of California, Los Angeles.

A few of the untreated plants had definite symptoms of root rot on January 20, and by February 28 all untreated plants were infected. On March 17 the foliage of all untreated plants was practically dead and the root systems were completely rotted, while neither root infection nor foliage symptoms could be detected in any plants of the treated series (Fig. 1). The experiment was then discontinued.

The perfect results obtained in this exploratory test with known infected material indicate that heat-treatment may safely be employed as an alternative to chemical treatment of infected rhizomes in the control of Phytophthora root rot of calla. There was no evidence of delay in either sprouting or blooming in this test comparable to that reported from chemical treatment.—A. W. DIMOCK, Cornell University, Ithaca, N. Y., and K. F. BAKER, University of California, Los Angeles, California.

Two New Records of Sphaceloma Diseases in the United States.—In July, 1943, diseased leaf specimens of *Aralia spinosa* L. from a nursery in northeastern Missouri were received from C. M. Tucker, of the University of Missouri, for identification of the fungus present. The severely diseased growth was affected by scab caused by *Sphaceloma araliae* Jenkins[1] (Fig. 1, A). On the advice of Dr. Tucker the entire planting of *Aralia* was removed from the nursery and destroyed by burning in order to avoid the spread of the disease from that source. Previously the scab had been known only from Maryland, where it was discovered at Edgewater, Anne Arundel County. The affected plants were in a wild plant garden where they had been transplanted from their native habitat. All of the diseased growth was removed and preserved as herbarium specimens. The disease did not recur. On his visits to the same county, that is, to Herald Harbor, which is about 10 miles from Edgewater, J. A. Stevenson has examined wild *Aralia spinosa* for scab, but none has been found. In 1941, using a culture isolated by the writer in 1934, Mr. Stevenson made a few inoculations on plants growing on his property at Herald Harbor. A limited number of lesions resulted on the inoculated foliage, which also was gathered for an herbarium specimen. As in the previous instance, no subsequent trace of the disease was found either on the inoculated or on nearby uninoculated plants.

Diseased willow leaves gathered in Skagit County, Wash., in August, 1943, by inspectors of the Bureau of Entomology and Plant Quarantine and referred to the writer for examination have been recognized to be affected by gray scab caused by *Sphaceloma murrayae* Jenkins and Grodsinsky (Fig. 1, B). (See Grodsinsky and Jenkins,[2] page 56, footnote 1.)

[1] Jenkins, A. E. New species of *Sphaceloma* on *Aralia* and *Mentha*. Jour. Wash. Acad. Sci. 27: 412–414. 1937.

[2] Grodsinsky, L., and A. E. Jenkins. *Sphaceloma murrayae* en diversas especies de *Salix*. Rev. Argentina Agron. No. 1, 10: 55–58. 1943 (issued March 15, 1943). The diagnosis of *S. murrayae*, just cited, is quoted from that appearing in the text of the article by Jenkins and Grodsinsky entitled "Sphaceloma on Willow in New Zealand" (Trans. Brit. Myc. Soc. Parts I and II, 26: 1–3. 1943. Issued April 8, 1943). Although the article in the "Transactions" was in press in advance of that in the "Revista," it was published slightly later as just indicated. For reasons of priority, therefore, the quoted diagnosis is the initial one.

The willow was identified by C. R. Ball as the Pacific Coast species, *Salix lasiandra* Benth. This is the first record of gray scab on this species of *Salix*, as well as the first from Washington State.

The hosts and geographic range of *Sphaceloma murrayae* as previously known[3] may be summarized as follows: *Salix babylonica* L., New Zealand; *S. fragilis* L., Rhode Island, New York, Virginia, and New Zealand; *S. fragilis* × *alba*, Virginia; *S. lasiolepis* Benth., California; *S. viminalis* L., Latvia, also on this species as cultivated in the Delta del Paraná, Argentina.

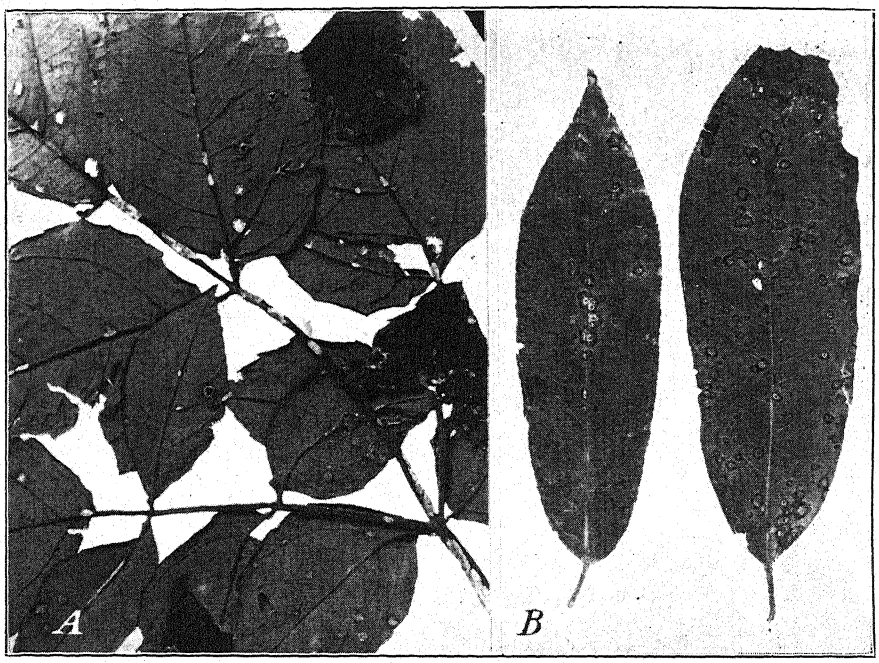


FIG. 1, A. Scab of *Aralia* on *Aralia spinosa*, northeastern Missouri, August, 1943. J. A. Denning. B. Gray scab of willow, on leaves of *Salix lasiandra*, Skagit County, Wash., August, 1943, gathered by inspectors of the U. S. Bureau of Entomology and Plant Quarantine. Photographs (×1) by R. L. Taylor.

Based on this distribution of *Sphaceloma murrayae*, Dr. Ball has furnished the following comment:

"It is interesting to consider the implications of three facts now apparent. 1. This fungus now is known to occur on five species of *Salix*, belonging to four Sections of the genus (Lucidae, Fragiles, Lasiolepes, and Viminales). Only the first two are closely related. 2. It has been collected on four continents, the countries represented being New Zealand, Latvia, Argentina, and the United States. In the last, it has been found in Rhode Island, New York and Virginia, in the East, and in California (S. W., Central, and N. W.) and in Washington, in the West. 3. Nearly all of the collection localities known to date are coastal and all are within the reach

[3] *Loc. cit.*, cf. articles cited in footnote 2.

of ocean influences. This may or may not be significant. These three facts, taken together seem to indicate that this fungus may occur still more widely than yet known, both as to host species and as to geographic range, especially coastal.''—ANNA E. JENKINS, U. S. Plant Industry Station, Beltsville, Maryland.

Experimental Control of Orange Decays with Thiourea.—Stem-end rots, caused by *Diplodia natalensis* and *Phomopsis citri,* and green mold, due to *Penicillium digitatum,* of citrus fruits have long caused serious economic losses. In preliminary experiments between January and July, 1944, thiourea has been highly effective in preventing the development of these organisms on midseason and late varieties of oranges such as Pineapple, Temple, Valencia, and seedling types. Before its commercial use on citrus fruit can be recommended, however, it will be necessary to have more complete knowledge relative to the possible effect on public health.

Fruit used in this work was either clipped directly from the trees or obtained from packing houses. Usually about 40 fruits were used for each treatment within an experiment, a number deemed sufficient because of the negligible amount of decay in treated lots in contrast to the large amount in checks. In most cases the fruit was exposed to ethylene vapors 40 to 50 hours before experimental treatment, because such exposure favors the development of stem-end decay. This gassing treatment assured a high percentage of decayed fruit in the check lots and at the same time served as a severe test of the efficacy of thiourea. To afford maximum opportunity for decay, the fruit was stored at 70° F. for 3 weeks before final readings were taken. Hence, it should be emphasized that the percentage of decay was considerably higher than is ordinarily obtained under commercial conditions.

In-vitro tests with thiourea dissolved in cornmeal agar (Difco, 1.9 per cent) showed that *D. natalensis* grew at 0.1 per cent but not at 0.2 per cent concentration and that *P. citri* and *P. digitatum* grew at 0.01 per cent but not at 0.02 per cent concentration.

Concentrations of thiourea covering a range from 10 per cent to 0.1 per cent were tested in several experiments. Approximately 50 per cent control was obtained with 1 per cent concentration and over 90 per cent control was obtained with 4 per cent (Table 1).

The solubility of thiourea is 9.18 gm. per 100 cc. at 13° C. In 164 fruits (4 experiments) dipped in 10 per cent thiourea at 21° C. there was 2.5 per cent decay; in 179 check fruits there was 30.7 per cent decay. Thus the 10 per cent concentration did not give a significant increase in control over that obtained with 4 per cent thiourea (Table 1). A 2 per cent solution was only slightly less effective than the 4 per cent.

A 5 per cent solution, which is well within the solubility range of thiourea, was arbitrarily chosen for extended tests. In seven lots of treated fruit (225 fruits) 1.8 per cent decayed; in the seven non-treated lots (222 fruits) 38.2 per cent decayed (Table 1).

One of the questions considered was the possible value of a wetting agent in the dip. Vatsol O.T., 0.05 per cent, was used, but later tests indicated that it gave no improvement in control. Nevertheless the use of a wetting agent was continued to insure more complete coverage.

Tests were made to determine the extent to which the effectiveness of thiourea is altered by washing or brushing the fruit subsequent to dipping. A greater degree of control was secured when the thiourea was dried on the fruit before washing. When the fruit dipped in [illegible] cent solution of thiourea was dried before washing there was no decay; when washed while still wet, there was 19 per cent decay, compared to 44.6 per cent decay in the check lots. Similar experiments were made in which fruits were dried by running them over 35 feet of revolving horsehair brushes. When the treated fruits were brushed while still wet only partial control was obtained (10 per

TABLE 1.—*Effect of various concentrations of thiourea on control of stem-end and green mold rots*

Concentration of thiourea	No. fruits			Fruits rotted	
	Total	With stem-end rot	With green mold rot		
Per cent				*No.*	*Per cent*
10	164	0	4	4	2.5
Check	179	38	17	55	30.7
5	225	3	1	4	1.8
Check	222	60	25	85	38.2
4	117	0	3	3	2.6
2	184	6	2	8	4.3
1	182	25	4	28	15.9
0.5	186	42	10	52	27.9
0.25	146	38	9	47	32.2
Check	184	61	5	66	35.8
0.10	147	45	10	55	37.4
Check	147	45	3	48	32.6

cent decay in the treated lots; 43.3 per cent decay in non-treated lots). Unfortunately, there were no experiments in which treated fruits were air dried before brushing. But in a single experiment with 80 fruits, excellent control was secured when the fruits were treated and air dried, after brushing. There was no decay in the treated lot; 49 per cent decay in the non-treated lot.

To prevent wilting of the fruit during storage several lots were waxed after the thiourea treatment by dipping them in a proprietary wax emulsion. It seemed desirable to test the effectiveness of thiourea combined with the emulsion. In two experiments with 252 Valencia fruits gassed 40 and 42 hours, there was no decrease in control when thiourea was applied in the water phase of the wax emulsion. The 5 per cent thiourea treatment resulted in 2.4 per cent decay and the 5 per cent thiourea plus wax emulsion, 1.2 per cent decay; and 34.5 per cent of the non-treated fruit decayed during 18 days of storage.

Several investigators have reported the causal organisms of stem-end rots

present in the "buttons" (calyx, receptacle, and part of pedicel) at picking time. After frequently observing the marked browning effect of thiourea on the "buttons," an experiment with 100 non-gassed fruits was made. Thiourea (5 per cent) caused browning of 48 per cent of the green buttons, while natural browning occurred on 16 per cent of the non-treated checks in 13 days. Ethylene likewise causes button browning but at the same time increases stem-end decay. It is obvious that browning *per se* is not a factor in stem-end rot control and that the decay-inhibiting effect of thiourea cannot be ascribed solely to its phytocidal effect on the "button" tissues. The marked increase in browning produced by thiourea is contrary to what might be anticipated from an anti-oxidant.

Some evidence that the linked sulfur of thiourea is important to its fungicidal activity was obtained from an experiment in which 69 gassed Valencia oranges were (a) dipped in 5 per cent thiourea, (b) dipped in 5 per cent urea, and (c) were not treated. At the end of 16 days there was no decay in (a), 56 per cent decay in (b), and 47 per cent decay in (c). Additional evidence that the linked sulfur is important was obtained from *in-vitro* tests. Media containing thiourea and media containing urea, in which the concentration of the $=C(NH_2)_2$ fraction was the same over a wide range of dilutions, inhibited growth of these fungi only when the linked sulfur atom of thiourea was present. In limited tests with several inorganic sulfur compounds no significant decrease in decay was noted.

Thiourea thus gave definite experimental control of stem-end rot and green mold decays of oranges. Although the data on gassed and on non-gassed fruit have not been segregated in this report, it is highly significant that thiourea is effective in controlling these decays even when applied after the fruit has been exposed to ethylene.—J. F. L. CHILDS, and E. A. SIEGLER, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

A Canker and Some Decay Fungi on Mimosa.—The mimosa tree (*Albizzia julibrissin* Durazz.) occurs abundantly in the southern part of the United States. Although a native of Asia, it is so well suited to the growing conditions encountered in much of our South that it has escaped from cultivation and now occurs in the wild at many places. This is the hardiest of the *Albizzia* species but it cannot be grown very far north of Washington, D. C. Even at Washington, it is so near the extreme northern limits of its range that it sometimes suffers from winter injury; and twigs and branches killed during the winter are common.

A recent examination of a number of mimosa trees in the District of Columbia disclosed numerous cankers (Fig. 1) on otherwise healthy branches and recalled an earlier collection made in 1935. Obviously many of the cankers, although present a number of years, have not caused progressive dying, and have resulted in relatively little damage. Smaller twigs

and branches were in some instances girdled but branches over an inch in diameter were seldom so severely affected. In each instance the canker had developed around the base of a smaller dead twig, indicating that the causal organism may be able to attack healthy wood only when it has been previously established in an adjoining dead portion. The fungus present in all cases was the ubiquitous *Nectria cinnabarina* Fr., for the most part in its imperfect stage (*Tubercularia vulgaris* Fr.). This fungus was isolated and obtained in culture from the dead wood of the cankered areas.

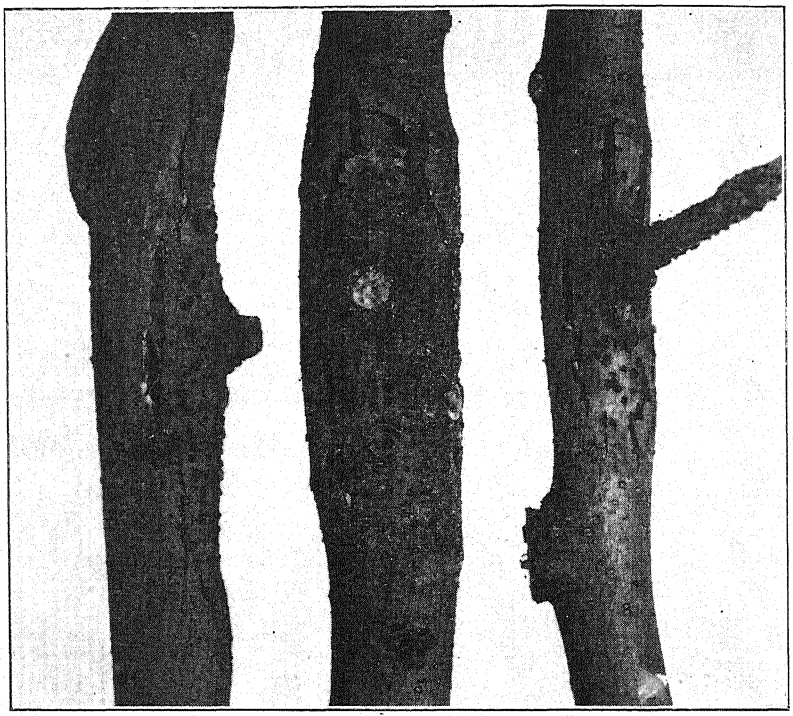


FIG. 1. Cankers on mimosa, each with a dead twig near its center and abundant fruiting of the *Tubercularia* stage of *Nectria cinnabarina* Fr. (Natural size.)

Nectria cinnabarina has been reported from a very wide range of woody plants, and from every continent in the world. To a considerable extent it is reported as a saprophyte on dead twigs and branches only, but in some instances, as in the present case, as a wound parasite associated with cankers following drought or winter injury. It has been studied particularly as an organism associated with senescence and decline of apple and other fruit trees and shrubs. Only two previous published reports of the fungus on *Albizzia* have been found: one by Dr. Alma Waterman (Plant Disease Reporter **23**: 85, 1939) from North Carolina and one by D. P. Limber (Plant Disease Reporter **16**: 138, 1932) in the *Tubercularia* stage from Virginia. Specimens have also been collected in South Carolina and Maryland in 1938 by members of the Division of Forest Pathology.

Although the *Nectria* was the predominant fungus noted, other fungi were present on dead and dying branches and doubtless were contributing to the decline of the host trees. Five species in particular were wood-decaying fungi: *Polyporus versicolor* Fr., *P. hirsutus* Fr., *P. tulipiferus* (Schw.) Overh., *Stereum albobadium* (Schw.) Fr. and *Schizophyllum commune* Fr. *Eutypella stellulata* (Fr.) Sacc. was also present in abundance on dead twigs and branches. All are common on other woody hosts, but do not appear to have been reported previously on *Albizzia*.—MARVIN E. FOWLER and JOHN A. STEVENSON, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland.

BOOK REVIEWS

BAWDEN, F. C. *Plant Viruses and Virus Diseases.* Second Edition. XI + 294 pp., 48 illus. Chronica Botanica Co., Waltham, Mass. 1943. Price $4.75.

The author deals almost entirely with those viruses that have received the most intensive studies, and, as in the first edition, emphasis is placed on the biochemical and physical studies. The literature lists contain approximately 640 citations, about 150 more than the first edition. Two cases are noted in which papers are mentioned in the text but not listed with the references, *i.e.*, on page 100, McKinney (1937) and page 140, Mulvania (1926). The book is well illustrated and the tables are understandable.

In Chapters 1 and 6 some of the author's views show a noticeable bias. His interpretations of plant-virus and plant-disease phenomena seem to be unduly influenced by traditional views and facts pertaining to the vertebrates. This is especially noticeable in connection with some of his views relating to virulence, vaccination, and acquired immunity. No mention is made of the sparing effect or interference manifested by some of the viruses of the vertebrates. In a book of this scope, this subject merits full discussion in relation to the unilateral interference or antagonism ("acquired immunity") which occurs between certain plant viruses. On page 97 the author seems to ignore completely the fact that the term *mutation* was in use long before it was employed in connection with the organisms that reproduce sexually. Attempts to preempt this term for the sexually-reproduced organisms are little short of pure sophistry.

Chapter 2, on external symptoms, strikes one as being rather brief for a book of this character. Chapter 3, on the internal symptoms, is brief, but the author has given a good résumé of the subject. Chapter 4, on virus transmission, is very brief, and there is a tendency to generalize—perhaps too much—from the studies on the tobacco mosaic virus. In table 3 it is not clear why the author regards the two species of true bugs, *Lygus pratensis* and *Piesma quadrata*, as leafhoppers.

Chapter 5 deals with viruses in relation to insect vectors. The author is critical of some of the interpretations put forward for the increase of virus in the leafhoppers, but it appears to the reviewer that the criticisms are essentially constructive. With reference to the author's discussion of Black's data on page 80, his statement, "it is noticeable that the number of successful inoculations is usually greater if the extracts of macerated insects is diluted 1/1000 than if diluted 1/100 or 1/10," seems not to be misleading as claimed by Kunkel in his review (Science 99: 450, 1944). Granting that the infective differences shown between the dilutions of 1/1000 and 1/100 are very small and probably not statistically significant, the fact remains that the complete data do give a strong indication that the virus was more infective in the insects when it was diluted. Bawden could have given more consideration to those of Black's data which do indicate increase of virus in the inoculated insects in the final tests. However, if there is a true decline in virus concentration in the inoculated insects after approximately the 12th to 16th day from inoculation, as thought by Black, it appears from his control-insect data that the insects which fed all their lives on infected plants contained an additional reservoir of virus that had been accumulating directly from the infected plants as indicated by Bawden. It appears that there is more to this problem than can be brought out in a brief discussion. Excessive condensing of subject matter in the book doubtless explains some of the author's statements which give rise to controversy. On page 76 the statement, "in published work there is no indication that vectors can ever infect healthy plants immediately after leaving infected ones," is a case in point. In the the interpretations being discussed time is a very important element, the word "immediately" therefore is not appropriate, for "immediately" is without time, and obviously time is required for all insect vectors to settle and begin feeding in a new location.

In Chapter 7 the author gives a very good résumé of serological methods, and of the results obtained with reference to plant viruses. He favors the use of the serological properties in virus classification, and in Chapter 14, on the classification of viruses, he gives rather serious consideration to the idea that these properties should be accorded essentially a generic rank.

Chapters 8 to 12 inclusive deal with the methods of virus purification, chemical and physical properties. The author has dealt with these fields very effectively. However, on page 140 he indicates that Mulvania in 1926 was the first to show "that tobacco mosaic virus could be precipitated by protein precipitants and resuspended without losing its infectivity." Mulvania's paper is not cited in the list of literature, but it seems probable that the paper referred to is the one published in Phytopathology 16, p. 583. This paper certainly does not support Bawden's statement. Mulvania used HCl, NaCl, NaOH, and glucose to determine their effects on the dializability of the virus through different grades of membranes, and he speculated on the possible protein nature of the virus, but he did

not mention precipitation or resuspension of the virus. Vinson and Petre, mentioned only casually by Bawden, were the first to record this and to show that the virus can be studied effectively by chemical methods. Stanley was the first to obtain virus in reasonably pure form and in large quantities for intensive study, and Bawden and Pirie were the first to show that the virus is a paracrystalin nucleoprotein and not a crystalin globulin. Furthermore, Bawden and his co-workers made important contributions in connection with the ultra purification of the virus.

Chapter 13, on the physiology of virus-diseased plants, is rather brief. While the literature on this subject is not very extensive, a more extensive résumé would have been welcomed. Chapter 14, on virus classification, covers the important proposals advanced by other investigators, and the author expresses his own views on the subject quite frankly. He seems to favor a system of scientific naming rather than a system of numbering, and he looks with decided disfavor on host reactions as a basis for classification. Chapter 15, on the control of virus diseases, is very introductory. Here the author seems to be somewhat out of his field. Chapter 16, on the origin and multiplication of viruses, is largely speculative. The author does not take the position that the viruses are definitely nonliving chemical entities. This is especially significant when it is considered that he has been actively engaged in advancing the study of viruses by physical and chemical methods.

This book contains much valuable information, and it should stimulate a critical viewpoint among virologists; for this the author deserves high commendation.—H. H. MCKINNEY, U. S. Bureau of Plant Industry Station, Beltsville, Maryland.

BAXTER, D. W. *Pathology in Forest Practice.* 618 pp., 232 figs. John Wiley and Sons, New York. 1943. Price, $5.50.

The problems of pathology in forest practice are discussed in the aspect in which they present themselves to the forester in the nursery, the plantation, the forest, the park, and in industry. Consequently little attention is paid to the small details of taxonomy, mycology, and etiology. Stress is placed on a working knowledge of the conditions which favor the development of various diseases and on the practical measures which can be taken to reduce their incidence or severity.

After a discussion of the nature of a plant disease, the causal fungi are briefly described. Emphasis is placed on the higher Basidiomycetes because they include the principal wood-destroying fungi. The various methods are given of determining loss and of making an appraisal of damage. Particular attention is given to critical relationships, such as (1) those of site and of cultural practice to the incidence of disease in the nursery, in plantations, and in the mature forest, (2) those of fungi and of certain other plant pests to disease in the mature forest, and (3) those of site and of care given shade and park trees to disease. Forest products are considered from the standpoints of decays, discoloration, stains, molds, and other defects.

The publication of this book is particularly timely, because during the war devastating inroads are being made on the nation's timber supply and because after the war many returned soldiers and war workers will find employment in extensive reforestation. Professor Baxter has assembled the information for those planning and directing the forestry programs so they can avoid costly and time-consuming errors. He has attacked a difficult problem in a masterful and authoritative way.

The printing is excellent both of the text and of 232 illustrations. A large number of literature citations appear at the end of each chapter. There is an excellent index.

Among technical books on forestry this one will appeal to practical men. It is considered indispensable for anyone who seeks to understand the conditions which favor tree damage by disease and with this information either to provide protection or to reduce the severity of the injury.—A. J. RIKER, University of Wisconsin, Madison, Wisconsin.

REPORT OF THE FIRST ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, BUREAU OF PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND, FEBRUARY 23 AND 24, 1944

The first annual meeting of the Potomac Division of the American Phytopathological Society was held at the Bureau of Plant Industry Station, Beltsville, Maryland, on February 23 and 24, 1944.

Research papers were presented on the mornings of February 23 and 24; the afternoon of February 23 was spent in discussing quarantine protection problems under the leadership of Dr. W. A. McCubbin; and a business meeting on the afternoon of February 24 was followed by a discussion on Wartime Work in Plant Pathology led by Dr. R. J. Haskell.

The following officers were elected: H. B. Humphrey, President; W. J. Zaumeier, Vice-President; T. F. Manns, Secretary and Treasurer; and H. P. Barss, Representative on the Council.

ABSTRACTS OF PAPERS PRESENTED AT THE FIRST ANNUAL MEETING OF THE POTOMAC DIVISION

Some Virus Diseases of Alliums. PHILIP BRIERLEY and FLOYD F. SMITH. Onion yellow dwarf is transmissible to and from wild garlic (*Allium vineale* L.). Garlic mosaic from late or Italian garlic received from Oregon is transmissible to onion and to *A. vineale* by sap and by *Myzus persicae* Sulz. Symptoms differ from those of yellow dwarf in both suscepts and the efficiency of transfer to onion is lower. Garlic mosaic in Spanish garlic has failed to transfer to healthy Spanish garlic, to *A. vineale,* or to onion in parallel tests by *M. persicae.* A mosaic of the ornamental *A. neopolitanum* Cyr. is transmissible to seedlings of this species by *M. persicae,* but not to onion in parallel tests. Aster yellows transferred from lettuce by *Macrosteles divisus* Uhl. induced yellowing and dwarfing in 3 of 10 Ebenezer onions in one trial, reproducing symptoms occasionally observed in field-grown onions that have been replanted in greenhouses for seed production.

Tobacco Disease Control by Rotation. E. E. CLAYTON. Crop rotation experiments on the control of soil-borne diseases affecting flue-cured tobacco were conducted from 1926 to date. The occurrence of the stem-rot and sore-shin diseases of tobacco, caused by *Sclerotium rolfsii* and *Rhizoctonia solani,* was not influenced by the cropping system. Continuous culture of sclerotium-susceptible crops did not increase tobacco stem-rot losses, nor did the use of immune crops reduce these losses. The occurrence of root knot and bacterial wilt caused by *Heterodera marioni* and *Bacterium solanacearum* was much affected by rotation practices. The organisms did not react the same. Root knot was effectively controlled by bare fallowing, while bacterial wilt was not. Again, once a field area was infected with wilt, successive crops of tobacco were uniformly and severely affected, while with root knot successive crops of tobacco planted on the same area might range from severely to slightly diseased. Effective disease control by rotation depended on the use of immune and not resistant crops. Over a period of years the results secured with resistant rotation crops were not superior to those obtained with susceptible. Fusarium wilt of tobacco, caused by *Fusarium oxysporum* var. *nicotianae,* appeared only where tobacco was grown in rotation with sweet potatoes. The time required at different locations varied from 2 to over 10 years.

Advances in Sugar-beet Seed Treatment. G. H. COONS and J. E. KOTILA. Improved stands have usually resulted from treatment of sugar-beet seed balls with fungicidal dusts prior to planting. Finely ground mercuric chloride (in equal parts with copper carbonate and urea), mercuric iodide, 2 per cent Ceresan, New Improved Ceresan, and other organic mercury compounds were superior to copper carbonate or cuprous oxide for seed treatment. Two per cent Ceresan used in equal parts with copper carbonate frequently showed more effective and lasting protection against damping off than 2 per cent Ceresan alone. Because of several factors, seed treatment for sugar beets, however, did not gain general adoption. Use of sheared sugar-beet seed in 1943 as sparse seedlings of the prevailingly single-germ seed units has revived interest in seed treatment because complete stands uniformly, but sparsely, distributed in the row are essential for mechanized thinning. Treatments effective with whole seed have been equally effective with sheared seed. Arasan and Spergon, used as dusts, also are effective for treating sheared seed, the latter

requiring heavier dosage than the usual recommendations. To obtain precision in planting sheared seed, pelleting of the seed is under trial. Standard pill-making techniques are used to coat the sheared seed in order to obtain a heavier seed unit, more uniform in size. Fungicidal treatments can be employed and nutrients and other substances can be added to the coating material, thereby increasing control of damping off.

The Response of Cantaloup-Cucumber Grafts to Inoculation with Fusarium bulbigenum var. niveum f. 2. C. E. COX. Cucumbers are not susceptible to the cantaloup wilt disease caused by *Fusarium bulbigenum* var. *niveum* f. 2. Grafted plants were used in order to determine whether, (a) cantaloup and cucumber plants growing on roots of the opposite species would respond differently to inoculation with this vascular parasite than plants on their own root systems, and also, (b) whether the shoots of one species would affect the susceptibility of roots of the other species. The following 4 types of grafted plants were prepared in the greenhouse: cantaloup scions on cucumber stocks, cucumber scions on cantaloup stocks, cantaloup scions on cantaloup stocks, and cucumber scions on cucumber stocks. The plants were grown in sand culture and watered with a nutrient solution. The roots of each plant were inoculated with 10 cc. of a macerated fungus mat from a liquid culture. The cantaloup-on-cantaloup grafts developed the symptom sequence characteristic of ungrafted plants. None of the cucumber-on-cucumber plants developed symptoms of the disease. In the plants composed of a cucumber scion on a cantaloup stock the stock developed typical symptoms and the scions withered and died. Cantaloup scions on cucumber stocks remained healthy. The extent of invasion of the parasite into the grafted plants was determined by culturing from various points both above and below the graft unions. The scion did not affect the susceptibility of the root stock to invasion by this fungus and the relative susceptibility of the scions was not altered by the susceptibility of the root stock.

Phytophthora Fragariae Hickman and Methods of Testing Strawberry Plants for Resistance. J. B. DEMAREE and W. F. JEFFERS. Testing strawberry plants for resistance to red stele has formerly been done in naturally infested soil. In order to improve this method a new technic for determining resistance by artificial inoculation was developed. The red stele organism is multiplied on Petri plates of lima-bean agar and the cultures are cut in small pieces and placed in water. Here zoospores are produced which are drained off and used as inoculum. Plants are grown in 2-inch pots and when sufficient new roots have grown are placed in a cool greenhouse. The plants are knocked from the pots, the bottom hole of the pot is plugged and 5 cc. of the zoospore suspension is added and the plant replaced. Susceptible plants show typical red stele symptoms in 2–3 weeks. Plants which do not become infected are reinoculated and if still resistant are set in naturally infested soil for fruiting tests.

The Fungicidal and Phytotoxic Behavior of Some Selected Organic and Inorganic Copper Compounds as Related to Weathering. M. C. GOLDSWORTHY. Copper malate, copper maleate, copper oxalate, copper citrate, copper tartrate, copper sodium polyphosphate, copper silicate, cupric oxide, cuprous oxide, and basic copper sulphate were tested. When the residues were subjected to weathering for 2 weeks, during which the rainfall was scanty, the compounds were lethal to the conidia of *Sclerotinia fructicola,* generally in accordance with the initial ionic copper content of the residues. Initial solubility was not related to fungicidal effectiveness. In no case were the conidia capable of changing the initial solubility or availability. In a few cases only, changes in solubility or availability accompanied onset of rainfall. In tests to determine the phytotoxic effect on Elberta peach leaves, those compounds that were initially soluble (though not fungicidal) exerted phytotoxic effects regardless of onset of rainfall. These compounds, as well as those not causing injury before onset of rainfall, became much more injurious when precipitation occurred. This indicated that environment played a considerable part in solubilizing the copper compounds so as to cause or increase phytotoxicity, but had little effect on their fungicidal behavior.

Virus Disease on the California Wonder Pepper in Delaware in 1943. J. W. HEUBERGER. Plants used were produced from two seed sources in plant houses used exclusively for peppers. In the field, those from one seed source averaged 54 per cent mosaic, whereas those from the other averaged less than 1 per cent mosaic. Since plants were set at the same time and fields were adjacent in many instances, chances for natural infection were equal. As no mosaic was reported in the plant houses, the striking difference in percentage infection may indicate a differential susceptibility to mosaic infection in the field. A new virus-like disease was also present, characterized by a continuous yellow discoloration beginning at the petiole and extending toward the leaf apex. These infected leaves readily fall from the plant. Fruits present at time of infection later have a yellow discoloration, often accompanied by pitting. Fruits formed after infection do

not develop. Plants from the same seed source with high mosaic infection likewise had high susceptibility to this tentatively named "Yellow Leaf" trouble. Culturing from all parts of infected plants yielded no causal organism. Preliminary transmission studies indicated that this trouble may be virus induced.

Oedema of Cultivated Violet Identified as Scab. ANNA E. JENKINS. "Oedema" or "wart" of sweet violet was first reported by B. T. Galloway in his "Commercial Violet Culture" (1889), which embodies the experience of Dr. Galloway and P. H. Dorsett in growing violets under glass at Garrett Park, Md. Recent examination of a specimen of the so-called oedema collected at Garrett Park by Galloway makes it seem certain that this actually represents what is now known as scab of violet caused by *Sphaceloma violae* Jenkins. Leaf symptoms are in general agreement with those of various specimens of the scab now available for comparison. Petiole lesions also correspond to those of violet scab, and scrapings from them show hyphal and conidial development of the *Sphaceloma*. The historic Galloway specimen is in the Mycological Collections of the Bureau of Plant Industry, Soils and Agricultural Engineering, where it was formerly entered in the "Physiological Collection." At the time when the "oedema" was giving trouble at Garrett Park, violet growers on the Hudson were contending with it, as the writer has been informed by a veteran violet grower. He has stated, also, as Galloway indicated, that conditions favoring the disease are poor ventilation, too much moisture, and cold. As previous reports show, the disease has been more or less widespread on native wild violets in the District of Columbia area; in 1935 it was found in great abundance on a wild violet growing extensively in a glade in Rock Creek Park, Maryland, *i.e.*, in the same stream valleys as Garrett Park. Galloway's account of "oedema," together with the specimen from Garrett Park, may now be construed as the first record of the *Sphaceloma* disease of violet.

An Undescribed Leaf Spot of Tift Sudan Grass. C. L. LEFABVRE. This leaf spot was first found in 1939 by Dr. G. W. Burton in a greenhouse at Tifton, Georgia, on plants of common Sudan × Leoti sorghum (now Tift sudan grass). Lesions on the leaves sent to the author varied considerably, the smaller ones being 0.5 to 1.0 mm. in each direction, while the larger ones measured up to 20 mm. in length and 2–6 mm. in width. The lesions were round to elliptical, the long axis parallel to the leaf veins, and oftentimes the spots seemed to be limited in their spread by the veins. Many of the lesions had coalesced so that comparatively large portions of the leaves were affected. The most characteristic features of the lesions, however, were the alternate light tan and darker, more narrow bands of tissue, resulting in a ringed or zonate appearance. Lesions were usually surrounded by a narrow reddish-brown border setting off the affected area from the healthy tissue. Symptoms on Tift sudan are strikingly similar to lesions on corn caused by *Helminthosporium maydis*. A species of *Helminthosporium* was readily isolated from the lesions on Tift sudan and when compared with *H. maydis* from corn, the former had spores averaging 61 μ and the latter 80 μ long. No difference in width was observed but there was a striking difference in the number of septa in the spores of the two fungi. Spores of the *Helminthosporium* from Tift sudan averaged 4.75 septa, while those of *H. maydis* from corn averaged 7.7 septa. Since these differences were found in the spores taken either directly from the hosts or from cultures, it appears the fungus from Tift sudan is not *H. maydis* but an undescribed species of *Helminthosporium*. From inoculation trials this fungus appears to be restricted to sudan grass.

Fungicides for Controlling Bulb Scale Rot and as Carriers for Growth Substances in the Propagation of Easter Lilies. W. D. MCCLELLAN and N. W. STUART. Bulb scales from some Easter lily clons are very susceptible to a rot caused by *Fusarium oxysporum* f. *lilii* Imle. Effective protectants are needed in scale propagation. The following materials have been tested in the greenhouses at the Plant Industry Station, Beltsville, Maryland: New Improved Ceresan (5 per cent ethyl mercuric phosphate), Spergon (98 per cent tetrachloro-para-benzoquinone), Arasan (50 per cent tetramethyl thiuram-disulphide), Fermate (ferric dimethyldithio-carbamate), Zincate (zinc dimethyldithio-carbamate), and formaldehyde. Best results were obtained with Arasan. Spergon and New Improved Ceresan also afforded good protection. Formaldehyde injured the scales. Fermate and Zincate were relatively ineffective. There was greater bulblet production and root and shoot development from the scales treated with Arasan than from those treated with New Improved Ceresan, Spergon, or formaldehyde. New Improved Ceresan inhibited bulblet formation and root development, but when the Arasan treatment followed Ceresan this inhibition was overcome. There were 300 scales in each treatment. The total bulblet production from those scales treated with Ceresan was 239; with Arasan, 442; the Ceresan-Arasan combination, 494; and from the untreated checks, 273. This stimulation did not occur when Spergon was used with the Ceresan treatment. When

Arasan or Spergon was substituted for talc as a carrier for the growth substances, naphthaleneacetic acid and indolebutyric acid, bulblet yields were greatly increased. The highest bulblet yields occurred when Arasan was used as the carrier for naphthaleneacetic acid. Scales treated with this combination produced 503 bulblets compared with 337 for naphthaleneacetic acid combination, 410 for Spergon alone, and 273 for the untreated checks.

Effect of Extracts from Cultures of Fusarium eumartii on Different Varieties of Potatoes. M. A. PETTY. Hot tap water (70° C.) was poured into 90-day cultures of *Fusarium eumartii* growing on sterilized whole oats. After 2 hours this aqueous extract was decanted. Half of this extract was steam-sterilized at 15 lb. pressure for 30 min. Excised vines of greenhouse-grown Irish Cobbler, Kasota, and President potatoes were placed in tap water (check), 50 per cent non-sterile extract in water, undiluted non-sterile extract, 50 per cent sterile extract in water, and undiluted sterile extract. Irish Cobbler vines in unsterilized extracts were noticeably wilted in 4 hours, while those of Kasota and President were unaffected. In 24 hours, only the few terminal leaves of Cobbler tops in the 50 per cent sterile extract were unaffected; all other leaves of plants in the extracts had completely collapsed, excepting the checks, which were erect. There was only a slight interveinal wilting and browning of the lower one or two leaves of the Kasota and President varieties in the unsterilized extracts, otherwise they were unaffected. There was no wilting of either of these last two varieties until after 36 hours: however, by this time there was considerable bacterial activity in the solutions. Excised tops of Rutgers and Beefsteak varieties of tomatoes were not affected by the extract.

Evidence of Hybridization between Physiologic Races of Ustilago hordei in Passage through Host. V. F. TAPKE. Seed of Odessa (C.I. 934) barley, highly susceptible to *Ustilago hordei* races 3 and 6, was inoculated with a mixture of chlamydospores of those two races in equal proportion. The content of physiologic races in 25 of the resulting smutted heads then was determined by inoculating differential hosts. Fifteen of the smutted heads contained only race 3, one contained only race 6, seven contained a mechanical mixture of both races, and two contained a different race capable of attacking varieties immune from race 3 or 6. This race appears to have resulted from hybridization. Further study has shown that the synthetic race is not a new one but produces reactions of the differential barley varieties similar to that of a naturally occurring race. A few of the further selections of the hybrid in later generations lost the capacity to attack varieties immune from race 3 or 6 but most of them have maintained it.

Tobacco Anthracnose, a New Tobacco Plant Bed and Field Disease. E. A. WALKER and SARAH W. MCINROY. Tobacco anthracnose was observed as a leaf spot in commercial plant beds on May 24, 1941, near Waldorf, Maryland. The following year it was very destructive to tobacco plants in poorly drained soils. Diseased plants placed in fields continued to develop symptoms under wet conditions. In the plant bed anthracnose was observed as a leaf spot, seedling blight, leaf midrib and petiole and plant stem cankers. In the field these symptoms extended as the plant grew and cankers were found on the branches of the flower head and on the seed pods. Anthracnose could be easily spread by means of contaminated seed. A *Colletotrichum* sp. was consistently isolated from tobacco leaf spots and stem cankers and its pathogenicity for tobacco established. A *Gleosporium* sp. was also obtained from diseased tobacco plants and from sectors of *Colletotrichum* sp. in culture. Spiral growth was observed with some strains of both organisms on some culture media.

Studies on the Virus of Brome-grass Mosaic. H. H. MCKINNEY. The end-point of virus activity in native extract is 10 min. when heated near 78.5° C.; is near 10^{-5} when diluted with water; in dry leaf tissue at laboratory temperatures the end-point is beyond 306 days. Grass hosts include wheat, rye, barley, oats, sorghum, Johnson grass, sudan grass, teosinte, corn, and other annual and perennial grasses in 9 of the grass tribes. In corn seedlings (2–3-leaf stage), at high growing temperatures, the first signs appear 36 to 40 hours after inoculation. The reactions consist of chlorosis, necrosis, and death of the host. In other grass hosts, the reactions range from carriers showing no visible symptoms, through mild to severe chlorotic mottling and streaking. Natural resistance increases rapidly as host plants age. The first leaves of garden bean var. Scotia develop small local necrotic lesions, the cotyledons of cucumber var. Early White Spine and the leaves of Samsun tobacco develop local faintly chlorotic spots and there is local increase of virus in each host. Experimental inoculations were accomplished by the carborundum wiping method.

SUMMARY OF THE PAPERS PRESENTED AT THE PLANT QUARANTINE SESSION

Introductory Review. W. A. McCUBBIN. It is encouraging that in its first meeting the Potomac Division should include a session on plant quarantine problems, thus recognizing understandingly that pathologists as a national organization have a responsibility in the field of foreign disease exclusion as well as toward national welfare and progress in teaching, research, and extension. This helpful interest is welcomed by the quarantine administration and is needed now, not only in the solution of immediate and postwar quarantine problems, but in the broader field of future national planning which should aim to establish the best permanent barriers against foreign disease introduction that science can devise, public opinion will support, and administration can maintain. Pathologists can contribute vitally to the future of our national plant quarantine system, especially in assuring that its various features rest on a sound biological basis. The national system of protection against foreign disease introduction may be considered as logically comprising four lines of defense,—the effort to secure extensive information on foreign pathogens as a requisite to effective understanding and planning; adequate control over the importation of pest-carrying materials in all its phases; domestic survey arrangements which would promptly disclose unfortunate disease introductions; and internal organization to deal with such introductions by eradication, by domestic state or federal quarantine action, or by control measures. Such a protective scheme contains many perplexing elements deserving of careful and earnest study by pathologists. In addition to the four chosen for discussion at this session, the foreign plant quarantine relations of the following merit much thoughtful attention,—nematodes; bacterial diseases; seed-borne diseases; foreign certifications; the importation of pathogen cultures; the value and limitations of inspection procedures; treatment possibilities; and the significance of races and strains of pathogens in the quarantine field. It is hoped that these and other aspects of the subject will come up for practical and profitable consideration in due course.

Quarantine Protection for Basic National Crops. HOWARD P. BARSS. Every enlightened government is impelled to include among its essential functions quarantine activities that aim to keep out of the country destructive plant diseases and pests which do not yet occur there. This is an integral part of the job of protecting agriculture, the primary safeguard of every nation against want. The danger of disease and pest introduction is real. For example, many hundreds of diseases are known to occur in other parts of the world which, like the Andean buba disease of potato, the Japanese brown rot of apple and pear, and a number of virulent grain rust races, have not yet reached North America; and everywhere nature is continually creating new strains of pathogens. Furthermore, disturbed world commerce coupled with expanded volume and speed of air traffic have tremendously increased the chances for disease introduction. Intelligent husbanding of agricultural assets is vital to permanent national welfare and demands that in providing defense against foreign pests special emphasis be given to those crops on which the country depends most. Since many phases of national interest are involved and quarantine funds and facilities are limited, some logical way is needed to establish a priority for crops which deserve special protective effort. A method for estimating crop importance has been suggested to me by W. A. McCubbin. He proposes a rating for each crop based on a summation of values assigned in each of several graded factors, namely crop use (food, feed, manufacture, soil-building, condiment, etc.); range of culture; quantity produced or money value; availability of domestic substitutes; and availability of foreign supplies. An estimate of the national importance of a crop thus obtained would rest on a broad base of facts, but other important factors cannot be disregarded in determining practical quarantine priorities. Each crop must be considered from the standpoint of the nature of its foreign pests and the adequacy of information about them, the relative cost and effectiveness of applicable quarantine measures, the effects of such measures on trade, the interests of producers and dealers, and the extent of public support. Decision as to the quarantine course to be recommended for the protection of any crop should rest primarily on a long-range view of the ultimate value of such a course to the country's agriculture. The decision will be modified, however, by the impact of such limiting factors as those just mentioned. Moreover, emphasis on the protection of major crops should not bar the protection of minor crops and other plant life where this appears feasible and in the national interest. The problem of adequate plant quarantine protection calls for the fullest utilization of existing knowledge and the aid of intensified research to provide a basis for intelligent action. Information is needed not only on foreign pathogens as such—their geographic and host ranges, race variations, life habits, and ecological relations—but also on their possible means of entry, their ability to attack domestic varieties, and their amenablity to treatment. In this field plant pathology has an indispensable service to render and its participation becomes more and more important as the destructive potentialities of foreign plant disease introduction and the value of defense against it become more generally recognized throughout the world.

Emphasis on the Study of Foreign Diseases. N. REX HUNT. Emphasis on the study of foreign diseases must be increased, owing to their probable wartime spread and the increased risk of introducing viable pathogens by fast air freight, if we are to meet our needs for information in promulgating and modifying quarantines; in finding diseases on material offered for entry or as soon as possible thereafter; in treating incoming material to eliminate risk of introduction of new diseases; in evolving eradication methods for diseases gaining temporary establishment in spite of quarantine vigilance; in providing treatments for control when eradication is impractical; in determining in advance of establishment of a foreign disease what the susceptibility of our commercial varieties and breeding stocks may be, so desirable resistant varieties may be supplied as promptly as possible when needed; in supplying data in advance of possible entry of a destructive disease of a major crop so agricultural planners may be prepared to maintain adequate supplies from other sources and to provide substitute crops for stricken areas. As aids in studying foreign disease problems we have indexes to the literature; the 600,000 records of diseases and insects found on incoming plant material are cross indexed by pathogen or pest, by host, and by country of origin and give a good idea of the types of pathogens and pests transported in commerce; and annotated lists of foreign diseases of important hosts are being prepared to supplement Stevenson's Manual of Foreign Plant Diseases which is now out of date. Plans for postwar Agriculture are being discussed. Provision for all needed work on plant diseases should be an integral and coordinated part of the plans. With respect to the study of foreign diseases, consideration should be given to the following: (1) The possibility of cooperative preparation and issuance in readily usable form of information already available regarding foreign diseases, and of keeping such information current. (2) Development of better coordination and wider use of correspondence and cooperative arrangements with foreign pathologists, with State Department representatives and with others abroad; of more effective arrangements for obtaining conferences with visiting pathologists from abroad; development of a general plan for stationing pathologists abroad temporarily, especially for the study of destructive diseases which cannot be studied here with safety and cannot be adequately studied through literature and by correspondence, in order that we may have these pathologists available for consultation here when needed; development of a comprehensive plan, in connection with the foregoing arrangements, for testing the reaction of American varieties and breeding stocks to foreign diseases. (3) Development of techniques to enable quarantine inspectors to detect foreign diseases, and of treatments to render incoming material safe for entry. At present most bacterial and virus diseases and thousands of fungous diseases in imported material are not determined, partly due to lack of techniques, partly due to lack of pure culture facilities; and for many foreign diseases no safe treatment is known to us. (4) Materialization of the plans made will require wholehearted public support based on a realization of the need for the work. Hence provision should be made for maintaining a constant and effective flow of information regarding the menace of foreign diseases, appropriate material to be supplied to agricultural, horticultural and gardening publications, to newspapers and magazines, to teachers in schools and colleges, to extension workers and any others needing it or able to use it effectively.

The Problem of Excluding Foreign Virus Disease. LEE M. HUTCHINS. Importation of living plants and parts from foreign countries is so intimately associated with improvement in agriculture, horticulture, and other plant industries, that it cannot conceivably be discontinued to alleviate the hazard of introducing dangerous plant diseases. The problem before us therefore is to determine what practicable measures will provide the greatest protection against the entrance and dissemination of these diseases in view of a continuing commerce in plant commodities. Exclusion of foreign virus diseases presents one of the most baffling aspects of this whole problem, complicated as it is by rapid airways transportation, fresh material shipped under refrigeration, symptomless carriers, and difficulties of detection in bulbs, tubers, rootstocks, cuttings, and dormant plants. Quarantine agencies need our full cooperation. Safeguards to be considered include: (1) knowledge of virus diseases occurring in foreign countries, to be obtained through literature, correspondence, travel and attendance at meetings, (2) appraisal of inspection, control, and quarantine methods as practiced abroad, and of relative hazards through plant shipments from the different countries, (3) stationing one or more of our pathologists at strategic locations abroad, to become familiar with virus diseases and keep us informed on current developments, (4) by cooperation with foreign governments and experiment stations, growing in foreign countries native American species of valuable trees and other plants and thus determining the diseases to which they are susceptible; in this way finding virus diseases dangerous to our species, before they reach our shores, (5) fumigation to free imported material of possible insect vectors, (6) evaluation of heat treatments and other methods of inactivating viruses in imported material, (7) detention and observation of questionable material under controlled growing conditions in

the United States before release, (8) prohibiting entry of certain material, where hazard of introducing a dangerous virus is known to be great, (9) through federal and state cooperation, inspection of imported plants where they are being grown in the United States, (10) inoculating the same or related species in America with inoculum from imported material, where this is warranted, (11) organizing virus symposia at national and international meetings of pathologists, and disseminating useful information on foreign virus diseases. An enormous reduction in importation of living plants, occasioned by world war, furnishes an unusual opportunity to improve protective measures against foreign diseases, for application at the cessation of hostilities and the resumption of normal commerce.

The Possibilities of Detention Procedures in the National Quarantine Scheme. R. KENT BEATTIE. Imported plants now spend but little time in transit to America. In the days of the sailing ship, weeks ensued, but the airplane has brought us to within 60 hours of the farthest parts of the world. The danger of the introduction of plant disease organisms in a living condition on imported plant material has correspondingly increased. In many countries, the plant diseases are entirely unknown. Commerce with these is rapidly increasing. Foreign inspection and certification, inspection, fumigation, and treatment of plant material at the port of entry are beneficial but only partially meet the danger. The port of first arrival for an airplane may be far within the interior of the United States. Many disease-producing agents are concealed in the tissues of the host with no external symptoms at the time of importation. They are out of reach of inspection and treatment. Especially is this true of such diseases as the Dutch elm disease, persimmon wilt, and some virus diseases when carried within the tissue of a resistant host. When a new disease enters it may destroy an established American crop or forest tree, or if controlled it may add greatly to the difficulty or expense of growing the crop or tree. Safety can be obtained only by growing introduced plants under control and observation until danger is past. This means reducing the quantity imported to the absolutely essential material and supplying adequate facilities for growing the imported plants isolated from American crop areas. Such isolation is already an established procedure in the control of animal and human diseases.

ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE THIRTY-SIXTH ANNUAL MEETING OF THE SOCIETY, CINCINNATI, OHIO, DECEMBER 9 TO 11, 1944

Physiological Maturity in Relation to Alternaria Blight in the Tomato. BARRATT, R. W. AND M. C. RICHARDS. Investigations show that all tomato varieties and breeding lines now available are susceptible to *Alternaria solani* when tested under conditions favorable for infection. Experiments over a three-year period to investigate differences in field defoliation show that the extent of defoliation of a tomato plant from *Alternaria solani* is related to the physiological maturity of the plant. Fruit to leaf quotient and period of yield are two of the major factors causing physiological maturity of the tomato. A relationship, which can be shown graphically, exists between the period of yield and period of defoliation regardless of variety. Early varieties, or early plantings of a variety, defoliate early because their periods of yield are early. The fruit load of a plant, as expressed by the fruit to leaf quotient, is correlated directly with defoliation, as shown in an experiment involving 16 varieties having a wide range in fruit load. The change in fruit to leaf quotient was followed during the season on four varieties by weekly samples. The data show that this quotient bears a relationship to the degree of defoliation in each variety. This relationship has been shown graphically.

The Use of Eradicant and Blossom Sprays on Sour Cherry in the Control of Brown-rot Blossom and Spur Blight Incited by Sclerotinia laxa Ader. and Ruhl. CALAVAN, E. C. Brown-rot blossom and spur blight of Early Richmond cherries has been controlled effectively in experiments in Door County, Wisconsin, during the past 2 years by the application shortly before budbreak of a copper-lime-monocalcium arsenite and fish oil, 3-2-2-(1½ pints fish oil)-50 eradicant spray, supplemented in early bloom by a protectant spray of Bordeaux mixture, 3-4-50. During the wet spring of 1943 this 2-spray program reduced the amount of total spurs blighted to 0.5 per cent, whereas check trees had 52.7 per cent spurs killed. Less satisfactory control was obtained by the use of either the dormant or the blossom spray alone. Indicated reductions in percentages of total spurs blighted were: for trees receiving the dormant spray only, 97.5; the blossom spray only, 82.1; and both sprays, 99.1. In 1944 the absence of infection periods until most petals had fallen resulted in a small amount, 2.8 per cent, of spurs blighted on check trees. Under such conditions the disease was checked 50 per cent by the dormant spray alone, 60 per cent by the blossom spray alone, and 92 per cent by the application of both sprays.

Wetwood of Elm in Illinois. CARTER, J. C. Wetwood of elm, under investigation in Illinois since 1939, has been shown by inoculation trials to be caused by an undescribed species of *Erwinia.* This bacterium has been derived consistently by cultural methods from the diseased trees studied. It produces gas by fermentation in the infected wood, with the development of abnormally high pressure in the trunk. The fermentation is accompanied by an accumulation of sap in abnormal amounts in the infected wood. The gas pressure, which may reach 60 lb. per square inch forces this sap out through branch cuts, crotch cracks, and other injuries, and produces what is commonly called flux. Sap in affected tissues is toxic; it causes foliage to wilt when it moves into the twigs, and produces a gray brown internal streaking in the current wood of branches through which it moves. Wilting and streaking can be confused with Verticillium wilt symptoms. Gas produced by fermentation in naturally infected trees contains approximately 46 per cent methane, 34 per cent nitrogen, 14 per cent carbon dioxide, 5 per cent oxygen, and 1 per cent hydrogen.

Chemical Treatments in Tobacco Seedbeds. CHAMBERLAIN, DONALD W. Better control measures are being sought for blackfire, wildfire, and damping-off in tobacco seedbeds. Experiments were continued at the Wisconsin Station in 1943 and 1944 with a number of different chemicals. Bordeaux mixture (3–4–50) has continued to give the best results, controlling both wildfire and damping-off 95–99 per cent. Home-mixed Bordeaux generally has given better control of wildfire than did the two popular brands of commercially prepared Bordeaux tested, although the latter satisfactorily controlled damping-off. Weekly treatments, beginning before the plants were above ground, were more effective than less frequent applications. Yellow cuprocide (1¾ lb.–100 gal.) controlled damping-off when applied as a weekly plant treatment or when mixed dry with sand (¾ lb.–100 lb. sand) and spread over the soil surface. Soil treatments, using Bordeaux powder, cuprous oxide, and Fermate, mixed dry into the soil before seeding, generally were unsatisfactory. Fermate, when applied before emergence of the plants, reduced stands in the seedbed.

A Brown Leaf Spot on Bromus inermis Leyss. caused by Pyrenophora bromi (Died.) Drechsler. CHAMBERLAIN, DONALD W., AND J. LEWIS ALLISON. *Pyrenophora bromi* causes a severe brown leaf spot on smooth brome grass in Wisconsin. The disease appears

early in the spring and develops best during moist, cool weather. Initial spring infection is by ascospores matured in early spring in perithecia formed on leaf blades of the host the previous summer. Conidia of the imperfect, or *Helminthosporium bromi*, stage of the fungus are produced sparsely during spring and summer but do not survive the winter and are the least important stage in the life cycle of the fungus. Optimum temperature for ascospore germination is 20° C., and for conidia 28° C. Perithecia bearing mature asci were produced in culture on potato-dextrose agar with either mono-conidial or mono-ascosporic isolates when incubated at 10° C. for 3 months. Inbred lines of smooth brome differ markedly in reaction to this leaf spot at Madison, indicating that selection and breeding offer a method of control. (Cooperative investigations between the Division of Forage Crops and Diseases, U. S. Department of Agriculture and the Wisconsin Agricultural Experiment Station.)

A Lethal Virus of Guar (Cyamopsis psoraloides DC.). CHESTER, K. STARR, AND W. E. COOPER. A necrotic, lethal virus disease was found causing an estimated 75 per cent loss in an experimental field of guar in Oklahoma. Symptoms appear in 6 to 14 days and comprise vein-clearing, sometimes a faint oak-leaf pattern, rolling, wilting, early stipple necrosis, and abscission of young leaves, terminal necrosis, necrotic stem lesions, yellowing and abscission of older leaves, marked stunting of growth, and ultimate death. Occasional plants show abatement of symptoms, following non-lethal necrosis, with mottling, leaf distortion, and stunting. In beans the virus causes systemic necrosis, but it differs from bean virus 4 in that it infects cowpea, soybean, mung bean, and petunia, and from tobacco ring-spot virus in not infecting *Nicotiana glutinosa* and in the type of symptoms produced on *N. tabacum* and petunia. The virus is transmissible mechanically, although not with perfect regularity. It is not destroyed by field temperatures of 42°–45° C. during many days, but the disease becomes masked in hot weather, and in the fall there is a spectacular return of symptoms. Natural infection of 0.1 per cent increases to 100 per cent in a few weeks.

Fusarium Seed-piece Decay of Potatoes. CUNNINGHAM, H. S. Many potato growers on Long Island have sustained serious losses because of seed-piece decay caused by *Fusarium*. Preliminary work indicated that the trouble was confined to seed stock obtained from one section and also, possibly, to one species of *Fusarium*. In 1944 seed-piece decay was found in lots of seed stock from two large seed-producing areas, and it now seems certain that two or more species of *Fusarium* can cause this trouble. The usual recommendation of curing seed pieces at high temperature and high humidity provides ideal conditions for growth of these Fusaria. Under such conditions the seed pieces can become 100 per cent infected and worthless for planting purposes. Seed treatment has been effective in reducing the number of infected seed pieces.

Effect of Nutrient Levels and Temperature on the Development of Puccinia graminis tritici. DARLEY, ELLIS F., AND HELEN HART. In wheat seedlings grown at low nutrient levels and inoculated with *Puccinia graminis tritici* a marked chlorosis accompanied uredia, sporulation was poor, and infection types were relatively low. At high nutrient levels there was almost no chlorosis, sporulation was good, and infection types were relatively high. In adult plants, a high nutrient level delayed maturity 1 to 3 weeks but scarcely affected reaction to stem rust. Mycelial development and sporulation in seedlings were best at 85° F., and poorest at 65° F. Both seedlings and adult plants of one of the Kenya wheats were susceptible to several races of stem rust at 85° F., but were resistant at 72° and 65° F. Newthatch seedlings were flecked by race 17 at 85° F. but seemed immune at 72° and 65° F.

Preliminary Report on a Potato Disease Closely Resembling Fusarium Wilt. DARLING, H. M. AND R. H. LARSON. During the past two years a striking and unusual potato disease has been under observation in Wisconsin. The first symptoms appear during the first part of July as an inward rolling and leathery texture of the lower leaves, followed by chlorosis. Early affected plants become rigid, most of the leaves roll inward, often turn brown, and die prematurely. Severe vascular browning of the lower stem may extend to the third node. Vascular browning of the roots and stolons is common. Tuber set on infected plants does not appear to be affected. A few tubers in a diseased hill become dull and abnormally soft and flabby, while others remain firm and bright. Axillary aerial tubers, tuber and stem necroses, excessive pigmentation, and rosetting were not observed. It does not appear, however, that the last two growing seasons have been such as to cause atypical symptoms of those wilt-producing Fusaria described in the literature.

Growth and Overwintering of Xanthomonas vesicatoria in Association with Wheat Roots. DIACHUN, STEPHEN, AND W. D. VALLEAU. Previous reports from this laboratory have demonstrated that *B. tabacum* and *B. angulatum* can form colonies on wheat roots, and can overwinter in soil, apparently in association with roots. In an extension of these studies *Xanthomonas vesicatoria, X. phaseoli* var. *sojense,* and *Bacterium medicaginis* var.

phaseolicola were found to form colonies on the surfaces of roots of wheat seedlings, and to a lesser extent on tomato, bean, and soybean roots. Also, *X. vesicatoria* was recovered from the roots of wheat plants growing out of doors in unsterilized soil infested with the organism. The soil was inoculated November 10, 1943. The bacteria were recovered in each of 5 trials between December 13, 1943, and March 14, 1944, but not on April 14. Individual plants were dug, the roots were washed in running tap water, crushed in water, and poured on the lower surface of water-soaked tomato leaves. Presence of the bacteria on the roots was revealed by development of leaf spots on the inoculated leaves. *X. phaseoli* var. *sojense* and *B. medicaginis* var. *phaseolicola* were not recovered during this period from roots of similar wheat plants growing in soil inoculated with these organisms.

Soil Treatment with Sodium Selenate for Control of Foliar Nematode of Chrysanthemums. DIMOCK, A. W. Excellent control of the foliar nematode disease of chrysanthemums was obtained by treating the soil with water solutions of sodium selenate. Six healthy cuttings, variety Yellow Fellow, were planted in each of 24 flats of composted soil on May 28, 1944. Twelve flats were given 25 parts sodium selenate per million of soil on June 8, and 6 of these were given 25 ppm. again on June 16. No further treatments were made. Inoculum, consisting of fragmented infected leaves, was distributed over the surfaces of all treated flats and 6 of the untreated flats immediately after making the June 8 treatment. All flats were watered overhead frequently until removed for examination on September 25. Superficial examination showed heavy and unmistakable infection in all 36 plants of the untreated, inoculated series, in only one plant of the 25 ppm. series, and in none of the 50 ppm. series. Infection had obviously spread to 3 flats of the untreated, uninoculated series. All plants of the 50 ppm. series showed considerable selenium injury of the lower foliage and some stunting, but the plants of the 25 ppm. series showed only slight selenium injury and little or no stunting.

Light, Drought, and Heat as Factors in Cotton Boll-Shedding. DUNLAP, A. A. The shedding of immature cotton bolls has long been recognized as a menace in obtaining maximum yields. Experimentally, shedding has been most readily caused by subjecting plants in actively-fruiting stages to periods (2 to 5 days) of low light intensities (50 to 2500 foot candles). Continuous wilting of plants for a few days also has resulted in excessive shedding, although frequent brief periods of wilting have not been effective. In addition, high daily temperatures above 100° F. have caused high rates of shedding. Association of these factors with low rates of photosynthesis has been ascertained by chemical analyses of leaf tissues. Certain varieties of upland cotton have shown considerable resistance to inadequate light effects, which predispose the plant to shed its fruiting forms.

Comparative Studies of Basidiospore Cultures of Rhizoctonia solani. EXNER, BEATRICE, AND S. J. P. CHILTON. It has been possible with certain tissue cultures of *Rhizoctonia solani* to produce basidial mats under relatively controlled conditions. The fungus is grown in flasks of potato-dextrose broth for about ten days, after which the mycelial mat is washed in distilled water and placed in a small Erlenmeyer flask with rooted cuttings of Alligator weed (*Alternanthera philoxeroides*). Sufficient water is added to maintain the cuttings and the humidity. Basidial mats form on the surface of the stems. Single basidiospore cultures isolated from these mats differ in rate of growth, size and shape of sclerotia, and in color. As many as 30 distinct cultural strains have been isolated from a basidial mat formed by one of these cultures. In limited tests no basidial mats were formed by single basidiospore cultures growing alone or paired in various combinations. These results indicate that the fusion nucleus presumably forms in the basidial stage of *R. solani*, is heterozygous, and that new strains can arise by segregation.

Temperature Inhibition of Storage Development of Net Necrosis and "Stem-end Browning" of Maine Potatoes of the Green Mountain Variety. FOLSOM, DONALD. In Northeastern Maine where Green Mountain potatoes are harvested as soon as possible after maturity, net necrosis (from current-season leaf-roll infection) and "stem-end browning" (cause undetermined) normally develop only in storage. Their development is maximum and most rapid at about 45° to 50° F., at which temperature the peak usually is reached in 60 to 90 days, and is not influenced by relative humidity. The optimum and maximum temperatures are somewhat higher for stem-end browning than for net necrosis. Development does not occur or is greatly reduced, even at optimum temperatures, if the storage temperature is first held for 60 days at 70° F., which is above the temperature range, or for 30 to 60 days at 32° to 36° F., which is near the lower end of the temperature range. This effect decreases progressively with shorter periods of exposures to the extreme temperatures and as the temperatures approach optimum for necrosis.

Potato Resistance to Leaf Roll. FOLSOM, DONALD, AND F. J. STEVENSON. In southwestern Maine, with leaf-roll plants of a commercial variety always in adjacent rows, 70 to 90 per cent of 8,586 seedlings tested in the six years from 1938 to 1943 acquired the

disease in one year, as evidenced by symptoms appearing the following year. Similarly exposed healthy plants of the Chippewa and Green Mountain varieties had respectively, 85 and 50 per cent leaf roll on the average per year. Among the 5,518 seedlings introduced from 1938 to 1941 inclusive, only 21 are left, most of which are from the cross Imperia × Earlaine. These 21 have remained practically free of leaf roll. Leaf-roll infection has been the basis for elimination of most of the others. When several of these apparently resistant seedlings were crossed with good commercial varieties, the resulting seedlings generally were more vigorous than the resistant parent, and considering all crosses for which they were used, only 55 per cent of these contracted leaf roll in the first year of exposure. It seems possible to produce commercially valuable new seedlings resistant enough to leaf roll to avoid severe field spread of the disease.

Control of Cabbage Mildew by Means of Benzene Vapor. FOSTER, H. H., AND J. A. PINCKARD. The production of cabbage seedlings for the early crop in Mississippi frequently is limited by *Peronospora parasitica.* Benzene vapor applied to experimental seedbeds resulted in an average of 256 seedlings per square foot; untreated beds produced an average of 158 plants. The average length of treated seedlings was 4.9 inches and of the untreated, 2.8 inches. Losses from undetermined root and stem rots continued to develop in the untreated plants. The most effective treatment consisted of nightly applications of 50 cc. of benzene applied to each cotton ball weighing 15 grams and suspended in the seedbed, one ball per 2 square yards. Wet seedbed covers having a thread count of 48 by 44 were used. Treatments were begun 36 hours after inoculation and continued from November 24 to December 28. Significantly effective control was obtained also after mildew appeared in farm beds using 50 cc. of benzene per square yard, three nights each week until the fourth true leaf developed. After the conclusion of the treatments and following cold wet weather mildew appeared in epidemic form, indicating the necessity of continued treatments until plants are set in the field.

Predisposition of Tomato Plants to Fusarium Wilt. FOSTER, ROBERT E. Under controlled conditions, using the dip-method of inoculating tomato plants with *Fusarium oxysporum* Schl. f. sp. *lycopersici* (Sacc.) S. & H., predisposition to development of wilt was brought about by several environal factors. In plants grown prior to inoculation at soil temperatures near the optimum range for growth (20°–28° C.), wilt developed more severely than in plants grown at lower or higher soil temperatures, regardless of the soil temperature maintained after inoculation. Wilt developed more severely in plants grown prior to inoculation in dry soil than it did in those grown in saturated soil or in soil with an optimum moisture content. Those plants grown in a short day prior to inoculation had a greater degree of wilt development than did those grown in a long day. Age of tomato plants at the time of inoculation had little or no effect on the subsequent development of wilt over the range tested (10–50 days old when inoculated).

Electrophoretic Studies with the Plant Viruses. FRAMPTON, VERNON L., AND WILLIAM N. TAKAHASHI. Leaf extracts obtained from healthy tobacco plants with ordinary buffers contain three proteins, as evidenced by the scanning patterns obtained with the Longsworth apparatus, and neither the concentration nor the chemical nature of these normal proteins is altered, so far as one can determine, by infection of the plant with tobacco-mosaic virus, the potato X and Y viruses, a latent potato virus obtained from the potato variety Katahdin, with James Johnson's cucumber mosaic No. 1, or Price's indicator strain to cucumber mosaic. Two proteins are extracted from the common bean and these likewise are not influenced on infection of the plant with the common bean mosaic or Zaumeyer's bean virus No. 4. The scanning patterns of the tobacco plant and potato tubers are not influenced by age in the time interval studied. In the instances of each of the viruses indicated, an abnormality appears in the patterns obtained from the infected plants and the location of the abnormality in the pattern in each case is correlated specifically with the virus in question. The appearance of the abnormality in the pattern of tobacco mosaic virus in tobacco plants is correlated in time with the appearance of the symptoms in the plant. The scanning patterns obtained with the tobacco, tomato, and potato plants are similar and are distinct from those obtained with the bean or cucumber.

Phylogenetic Relationship of the Nine Known Leaf-hopper Vectors of Pierce's Disease of Grape. FRAZIER, NORMAN W. Three species of leaf hoppers, *Draeculacephala minerva* Ball, *Carneocephala fulgida* Nott., and *Neokolla circellata* (Baker), have previously been reported to transmit the virus causing Pierce's disease of grape. More recent work has shown that 6 additional species of leaf hoppers, *Carneocephala triguttata* Nott., *Helochara delta* Oman, *Neokolla gothica* (Sign.), *N. confluens* (Uhler), *N. heiroglyphica* (Say), and *Cuerna occidentalis* Oman and Beamer, also are able to transmit the virus. All 9 species are contained in the single subfamily Amblycephalinae, members of which are commonly known as sharpshooters. Every species of this subfamily thus far tested has proved to be a vector of the virus. Many species of leaf hoppers in other subfamilies

have been tested and not one has proved to be a vector. The present evidence strongly suggests that a phylogenetic relationship exists between the Amblycephalinae and the ability to transmit the virus.

A Pythiaceous Stem-end Rot of Potatoes. GOSS, R. W., AND J. H. JENSEN. In the 1943 potato crop a tuber rot was found with symptoms similar to those described for "leak" except that the rot usually occurred at the stem end instead of centering around wounds and usually was present when the tubers were dug. A pythiaceous fungus was isolated, which, when added in a water suspension to soil in which potato plants were growing, infected roots, stems, stolons, and tubers. Some tubers were completely rotted when dug and others had "leak" symptoms at the stem end ascribable to infection from the stolon. Infection resulting from soil inoculation was greater at 22° than at 30° C. and with high rather than low soil moisture. Isolates tested by wound inoculation of tubers produced a rapid rot at 20° to 30° C. The maximum development of the rot was at 25°, whereas only a slight amount of rotting occurred at 5° to 10° and at 35° C.

Influence of Rate, Depth, and Time of Planting Spring Wheat on the Incidence of Root Rot. GREANEY, F. J. Field experiments to determine the effect of rate, depth, and date of seeding spring wheat on the incidence of root rot caused by *Helminthosporium sativum* and *Fusarium* spp. were made at Winnipeg, Manitoba, from 1936 to 1943. In 1936, 1937, 1938, and 1939, plantings of wheat were made at 4-day intervals during May and early June (10 plantings). Each year the effect of planting time on plant emergence, yield, and incidence of root rot was constant, the severity of seedling blight and root rot increasing and percentage of plant emergence and yield decreasing with lateness of planting. In experiments from 1939 to 1943 root rot severity increased with thickness and depth of planting. Further work is necessary to elucidate the factors responsible for variations in the incidence of root rot in relation to rate, depth, and time of planting under local conditions. Soil temperature and moisture are of outstanding importance, influencing both yield and root-rot development. In the meantime, planting spring wheat not too thickly or deeply, and at the earliest feasible date, is evidently a practical method of reducing losses from common root rot in Manitoba.

The Balance of Calcium and Potassium in Relation to Club Root of Cabbage and Potato Scab. GRIES, GEORGE A., JAMES G. HORSFALL, AND H. G. M. JACOBSON. The calcium–potassium ratio as applied was varied widely at three different pH levels, acid [with $CaSO_4$], medium [with $CaCO_3$], and alkaline [with $Ca(OH)_2$]. The same experimental design was used on two fields, one sandy, the other loamy. At any given Ca–K ratio, both club root and scab followed the expected relation to pH. In both fields and for all Ca sources, scab increased as the Ca–K ratio was reduced by increasing the K and holding the Ca constant. This occurred although the pH remained constant within each Ca series. After reaching a peak, scab declined with a further decrease in the Ca–K ratio effected by holding the K at a high level while decreasing Ca. This decrease in scab occurred either at a constant pH or in a range where scab might have been expected to increase. In the low-K sandy soil, the peak was at a higher Ca–K ratio than in the relatively high-K loam soil. Except at very high Ca–K levels, the curve for club root ran a mirror image to that for scab which suggests that the effects of the Ca and K balance are inverse with the two diseases. Probably one of the effects of pH is to alter the Ca–K ratio by altering the Ca-ion concentration in the soil.

Soil Treatments and Apple Replant Survival in Xylaria mali–Infested Locations. GROVES, A. B. Black root rot of apple is widely distributed throughout the Cumberland-Shenandoah fruit-growing region and is a major factor in tree loss. The survival of replants made in locations from which diseased trees have been removed is too low to justify such replanting. Soil treatments have been resorted to in an attempt to improve the chance of replant survival during the critical early years in the life of the tree. Observations of replants growing in treated locations have now been completed for 7 seasons. A carbon bisulfide treatment appears most promising to date, with manure perhaps the poorest. Results with urea and chloropicrin treatments show promise but cannot be considered more than preliminary. Results with other treatments are less indicative.

Compatibility of Organic Fungicides and Summer Oil. GROVES, A.B. There is a most pressing need for a fungicide compatible with summer oil for use on apples where the use of an ovicide is regarded as essential to codling moth control. The standard sulphur and copper fungicides available are either not compatible with oil or are unsafe at the season required. Organic fungicides have been used on the York variety both prior to, and in combination with, summer oil. When used in combination, severe injury resulted with Compound 604, moderate injury with He-175, and light but characteristic injury with Chloranil and Puratized N5X. No injury resulted with Compound 341 or its hydrochloride form, or with Fermate. The 10-day-old residues of all organic materials proved compatible with subsequent oil applications.

Tests with New Organic Fungicides on Orchard Fruits. HAMILTON, J. M., D. H. PALMITER, AND G. L. MACK. McIntosh trees sprayed with Puratized N5X, ½ pt./100, had 2 per cent scabby fruit as against 22, 24, and 44 per cent for Isothan Q15, 1 pt./100; Fermate, 1–100; and Micronized sulphur, 5–100, respectively. Puratized N5X has the eradicative property of lime-sulphur without causing visible injury. It did not control cedar-apple rust. Q15 is also eradicative. It gave excellent control of brown rot on sweet cherries. Apparently because of poor tenacity, Q15 did not adequately control cherry leaf spot, sooty blotch and leaf blight of pear, and cedar-apple rust. Experiments on potted apple trees in the greenhouse corroborate the field data. Puratized N5X and Q15 are fungistatic, with moisture and the time element as factors in their effectiveness. Dithane alone is somewhat comparable to Fermate for control of apple scab and cedar-apple rust. Unlike Fermate, it did not control peach leaf curl or brown rot on sweet cherries. Dithane is incompatible with lead arsenate and nicotine sulphate. The arsenical mixture severely injured Cortland foliage, russeted McIntosh fruit, and defoliated Montmorency cherries. Dithane added to a zinc sulphate–lime mixture is more effective and compatible with lead arsenate. Indications were that Dithane, ¾–100, added to a zinc sulphate–lime mixture, 1–½–100, will control cherry leaf spot.

Relation of Physiologic Races of Puccinia graminis tritici to Wheat Improvement in Southern Mexico. HARRAR, J. G., W. Q. LOEGERING, AND E. C. STAKMAN. In physiologic race surveys of *Puccinia graminis tritici* made in Southern Mexico for 12 years, only races 38, 59, and 19 have been sufficiently prevalent to be of practical importance. Although these races have been generally distributed, there has been some localization in prevalence within the region. Races 56 and 17, which are very prevalent in Northern Mexico and the United States, have been found only occasionally in Southern Mexico. During the past two years many wheat varieties and hybrid lines have been tested under epidemic conditions near Mexico City. As would be expected, Marquis and certain other varieties that are completely susceptible in the spring-wheat region of the United States were highly resistant to stem rust. Rival, Newthatch, Regent, Renown, Pilot, Mida, and certain other varieties recently produced in the United States and Canada were almost free from stem rust and highly resistant to leaf rust, but all matured two or three weeks later than certain early but very susceptible commercial varieties commonly grown in Mexico. Appropriate crosses, therefore, are being made to combine earliness with rust resistance. (Cooperative investigations between the Secretaría de Agricultura y Fomento de México, the Rockefeller Foundation, the U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Varietal Variation and Inheritance Studies on Natural Water-soaking in Tobacco. HEGGESTAD, HOWARD E. Natural water-soaking in several foreign, domestic, and local varieties of tobacco was compared under moist-chamber and outdoor-seedbed conditions. The percentage of plants water-soaked varied from 100 to 8; and a score, based on leaf area water-soaked, varied from 2.36 to 0.09. Crosses between several susceptible and resistant varieties indicate that resistance may be partially dominant in the F_1. The F_2 and F_3 generations clearly indicate segregation of genetic factors. Some F_3 lines approach the resistance and the susceptibility of the parents. The inheritance of natural water-soaking appears to be governed by multiple factors. Water-soaked plants in several series of tests were atomized lightly with the wildfire organism. One moist-chamber test yielded an average of 18.5 lesions per plant on one variety as compared to 3.9 lesions on another. Following water-soaking, scores of 2.47 and 0.67, respectively, were obtained on these same varieties. Varietal infection in tobacco seedbeds varied from 34 to 1.2 lesions per leaf, relative susceptibility to the disease being correlated with relative susceptibility to water-soaking. Varietal variation in water-soaking was found in tomatoes, oats, and corn. Inoculation with the halo-blight organism on oats exposed to natural water-soaking yielded results similar to those on tobacco.

Growth of Tobacco Seedlings Stimulated by the Addition of Peanut-hull Meal to the Plant-bed Soil. HENDERSON, R. G. Treatment of tobacco plant-bed soil with urea and calcium cyanamid for the control of weeds leaves the soil toxic to plant growth for several weeks. In preliminary tests the addition of organic matter to treated soil, in order to stimulate the growth of microorganisms, quickly brought the soil back to productive state. In an extensive test at Chatham, Virginia, in 1943–44, treated soil amended with 2 lb. of peanut-hull meal per square yard produced more vigorous plant growth and a larger number of usable tobacco plants than did treated soil left unamended. Analyses of soil samples taken 41 days after the peanut-hull meal was added revealed no significant differences in pH or ammonia-nitrogen, nitrate-nitrogen, and nitrite-nitrogen content between the amended and unamended plots. No analyses were made after the 41st day. The growth stimulation from the use of peanut-hull meal probably was due in part to improvement of the physical condition of the soil, since some stimulation was noted also on plots where neither urea nor calcium cyanamid was used. From these data it appears

that the benefits obtained from treating soil with urea and calcium cyanamid can be increased by adding organic matter.

Zinc Dimethyl Dithiocarbamate and the Control of Early Blight and Anthracnose on Tomatoes and of Leaf Hoppers and Early Blight on Potatoes. HEUBERGER, J. W., AND D. O. WOLFENBARGER. In experiments on tomatoes and potatoes, designed to determine the protective value of organic and inorganic fungicides, zinc dimethyl dithiocarbamate was an outstanding material. In all tests the fungicides were used at a concentration of 1.5–100 active ingredient. The percentages of control of early blight of tomato on Sept. 28 were 75 for Bordeaux, 71 for zinc dimethyl dithiocarbamate, 66 for Compound A, 52 for Fermate, and 24 for the untreated; the percentages of tomato anthracnose infection at the September 28 harvest were 0.5 for zinc dimethyl dithiocarbamate, 9.3 for Bordeaux, 10.4 for Compound A, 18.0 for Fermate, and 25.3 for the untreated. The percentages of control of early blight on Sept. 27 on late-planted Dakota Red potatoes was 97 for Bordeaux, 92 for zinc dimethyl dithiocarbamate, 90 for Compound A, 80 for Fermate, and 65 for the untreated. The Ca, Na, Fe, Cu, and Pb dimethyl dithiocarbamates were less effective fungicides than was Zn dimethyl dithiocarbamate. On early-planted Irish Cobbler potatoes the percentages of control of potato leaf hoppers (*Empoasca fabae* Harr.) were 50 for zinc dimethyl dithiocarbamate, 38 for zinc sulphate–lime (1–$\frac{1}{2}$–100), 34 for Bordeaux, 28 for Compound A, and 16 for the untreated. Adding zinc sulphate–lime (1–$\frac{1}{2}$–100) to organic and inorganic fungicides increased leaf-hopper control by approximately 20 percentage points for each material. Yield records were not complete when this abstract was submitted.

Preliminary Report on DDT in the Potato Fungicide Program. HEUBERGER, J. W., AND D. O. WOLFENBARGER. In an experiment comprising 44 materials on late-planted Dakota Red potatoes, DDT (dichloro diphenyl trichlorethane) at a concentration of $\frac{3}{4}$–100 was used alone and in combination with the two fungicides, Compound A (copper oxychloride) and zinc dimethyl dithiocarbamate, in a 5-application spray schedule. Single row plots 20 feet long were used in a replicated, randomized block design. Potato leaf hopper (*Empoasca fabae* Harr.) counts on September 8 averaged approximately 7.6 per 20-foot row for those treated with fungicides and those untreated, and averaged 1 for DDT alone or combined with Compound A and zinc dimethyl dithiocarbamate. DDT had no effect on control of early blight (*Alternaria solani*) when used either alone or in combination with the fungicides. Where DDT was used alone or combined with the fungicides, the plants were taller, broader, darker green in color, and had larger leaflets; also, the leaflets were flatter (less cupped) than when untreated or when the fungicides were used alone. No foliage injury was observed where DDT was used. The border effect of DDT was pronounced, as replicates of other treatments that fell next to the DDT had characteristics of the DDT plots. No yield records were available when this abstract was submitted.

The Cherry Virus Complex in New York. HILDEBRAND, E. M. Several nonlethal abnormalities of virus or genetic origin occur on cherries in New York. The graft-transmissible nature of certain elements in this complex was first demonstrated in 1936. Subsequently four virus diseases have been distinguished in the complex on sour cherry—yellows (*Chlorogenus cerasae*), ring spot (*Annulus cerasae*), green-ring yellows, and rosette—and two on sweet cherry—tatter-leaf and mottle. The seed-transmissible crinkle on *Prunus avium* appears to be of genetic origin. The several cherry viruses are transmitted readily by grafting and produce distinctive symptoms. On Montmorency the incubation periods of ring spot and rosette are short (± 2 weeks) and that of yellows and green-ring yellows is long (± 1 year) whereas on Black Tartarian that of tatter-leaf virus is short (± 2 weeks) and that of mottle is long (± 1 year). When indexed on peach, ring spot ordinarily is distinguished by necrosis followed by recovery, whereas yellows induces (1) rosette and stunting, (2) mosaic, or (3) no symptoms. When indexed on Italian prune, yellows (strain 1) and sweet-cherry mottle induce symptoms simulating prune dwarf. The fact that certain of these viruses have been located in the rootstocks and grafted trees in nurseries and also in the wild (*P. avium, P. virginiana, P. pennsylvanica*) suggests their probable origin.

Effect of Crown-gall Bacterial Metabolites, Crown-gall Tissue Extracts, and the Composition of the Medium on Growth in vitro of Excised Tobacco and Sunflower Tissue. HILDEBRANDT, ALBERT C., A. J. RIKER, AND B. M. DUGGAR. In connection with crown-gall studies, the effects of various modifications of the culture medium on the growth *in vitro* of excised tobacco and sunflower tissue were observed. White's synthetic medium was used with various supplements. When fermented media from both the virulent and attenuated cultures, respectively, of *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.* were added, slight stimulation of tobacco tissue occurred at the lower concentrations of the fermented material. A slight inhibition was found at the higher concentrations. Fermented media from the attenuated strain strikingly inhibited sunflower

tissue at all except the lowest concentrations. Extracts from crown galls on marigold stimulated growth of both tissues at all concentrations; Paris daisy gall extract similarly favored the sunflower tissue, and yeast extract the tobacco tissue. Tomato gall extract and yeast extract, respectively, stimulated sunflower tissue at low concentrations but inhibited growth at higher concentrations; tomato gall extract similarly affected tobacco tissue. Paris daisy gall extract inhibited growth of tobacco tissue at all except the lowest concentrations. Varying concentrations of important mineral salts and vitamins were studied in 120 different combinations involving 22,000 tissue transplants. These have indicated what happened when the basic medium was altered. Improved media were developed.

Some Ontogenic Characteristics of Ustilago linearis forma hordei. HIRSCHHORN, ELISA. Since collections of *Ustilago linearis* studied by Osner, Davis, and Fischer differed physiologically, morphologically, and pathogenically, certain ontogenic characters of forma *hordei* of *U. linearis* were studied in plants of *Agropyron trachycaulum* (Link) Malte growing in the greenhouse. Soon after infection, hyphae reach the base of the coleoptile. Within 10 days the mycelium can be found between and within parenchyma cells and in the vascular tissues, being especially conspicuous near the xylem vessels and near nuclei of the host cells. Actively growing hyphae have long, binucleate cells. Within 6 weeks vegetative host tissues are fully invaded, and chlamydospores begin to form in terminal cells of hyphae growing in the parenchyma between the vascular bundles. Caryogamy occurs just before or at the time echinulation begins. Echinulations of the young epispore shrink as the chlamydospore matures. Mature chlamydospores are uninucleate and have a single membrane, the epispore.

Fungicidal Action of Reagents for Amino Acids, Amines, Aldehydes, and Other Reactive Cell Constituents. HORSFALL, JAMES G., AND GEORGE A. ZENTMYER. Zentmyer's theory (Phytopath. 33: 1121, 1943) that 8-hydroxyquinoline is fungistatic because it precipitates essential metals in the spore suggested that other reagents for metals or reagents for other products of intermediary cell metabolism might also be fungistatic, possibly fungicidal. The following reagents for essential metals were previously known or are here shown to be fungistatic: "cupferron," K-periodate, resorcinol, Na-diethyl dithiocarbamate, thiourea, thioglycolic acid, hydrogen sulphide, alizarine, benzidine, α-benzoin oxime, *o*-benzoquinone dioxime, catechol, cinchonine, aniline, dithio-oxamide, malonic acid, nitrobenzene-azo-α-naphthol, phenylhydrazine, K-ethylxanthate, quinalizarin, salicylaldoxime. Similarly, reagents for amines or for amino acids that are fungistatic are: ammonium rhodanilate, *p*-bromo benzenesulphonyl chloride, 3-5 dinitrobenzoyl chloride, *m*-nitrobenzene sulphonyl chloride, *p*-nitrophenyl isocyanate, Na-3-naphthoquinone-4-sulphonate, *p*-toluenesulphonyl chloride, Reinecke salt. Fungistatic reagents for aldehydes are: α-benzyl-α-phenylhydrazine hydrochloride, dimethyldihydroresorcinol, 3-5 dinitrosalicylic acid, α-naphthol, α-naphthylhydrazine hydrochloride, phenylhydrazine, benzenesulphohydroxamic acid. A few reagents for these groups were nonfungistatic.

Derivatives of Pyridine and Quinoline as Cationic Fungicides. HOWARD, F. L., H. L. KEIL, AND H. H. MOSHER. Monobasic acid derivatives of pyridine, picolines, lutadines, collodine, quinaldines, quinoline, and isoquinoline have been developed that exhibit high fungitoxicity, low phytotoxicity, and surface activity as desired. Examples of soluble cationic forms are cetyl isoquinolinium bromide, lauryl pyridinium thiocyanate, lauryl quinaldinium bromide, and lauryl isoquinolinium acetate, which follow the general pyridine ring structure (see following abstract). These compounds are fungitoxic and stable over a wide pH range (pH 3 to pH 9), commercially available from inexpensive noncritical materials, and reportedly not poisonous to higher animals. Assays of fungitoxicity to spores of *Macrosporium sarcinaeforme* have given mean LD50 values of 0.5 ppm. to 20 ppm. and mean LD95 values of 1.5 ppm. to 50 ppm. when the data are plotted on logarithmic-probability paper. Phytotoxicity determinations on tomato, bean, apple, and potato foliage indicate a considerable margin of safety. Toxicant concentrations of 1:5000 seem adequate as foliage sprays and as antiseptic fruit washes. The compounds are compatible with insecticides and adjuvants, except soaps. The wetting property, solubility, and resistance to weathering can be varied to meet specifications by altering the molecular structure.

Nicotinium Derivatives as Fungicides. HOWARD, F. L., H. L. KEIL, L. WEIL, AND C. F. WOODWARD. Twenty-five non-metallic derivatives of nicotine, in which an alkyl, aralkyl, or substituted aralkyl radical (R) and an acid radical (X) were attached probably to the nitrogen in the N-methyl pyrrolidine ring, have been prepared and tested for fungitoxicity, phytotoxicity, and surface activity. Laboratory assays of toxicity to *Macrosporium sarcinaeforme* spores have shown general relationships between the molecular structure of the radicals added and the fungitoxicity. Lethal dose (LD50) values can be varied from 11 ppm. to 2000 ppm. and LD95 values from 23 ppm. to greater than 3000 ppm. by attaching different, R and X, radicals to the N-methyl pyrrolidine nitrogen atom.

There is no correlation between fungicidal value and nicotine content or molecular weight. Derivatives having benzyl and short-chain alkyl (R) radicals exhibit much lower fungitoxicity than those having lauryl or cetyl (R) radicals. The margin of safety between fungicidal action and injury to foliage can be estimated by comparing LD50 values for fungitoxicity with LD50 phytotoxicity values; *i.e.*, the mean dose by which 50 per cent of tomato foliage is killed within 5 days after having been immersed for 5 seconds. The mean LD50 fungitoxicity value of benzyl nicotinium chloride was 210 ppm. and of lauryl nicotinium oleate 11 ppm., while the mean LD50 phytotoxicity values were 20,000 ppm., and 4000 ppm. respectively.

Water Influencing Host-predisposition. JOHNSON, JAMES. Efforts were made to separate and measure the "forms" of water affecting host-predisposition to disease. High atmospheric humidity, plant-surface water, and water-content of the plant cells themselves are not determining factors in host predisposition to many diseases except as these conditions influence a fourth form of water favoring invasion. Little information exists relative to excessive water in the intercellular spaces and vascular tissues, the extreme of which is known as "water-soaking." The more inclusive and descriptive term "water-congestion" is suggested. Water-congested tissues may be visible only by magnification, or the spaces may be only partly filled with water and not demonstrable. Determinations of the total water content of tissues on a variety of species were made throughout the growing season during varying weather conditions. By this method, increases attributable to water-congestion were not significant. The total water-content of 50 species varied from 59 to 96 per cent, but each remained fairly constant through varying periods of weather and predisposition to disease. Artificially induced water-congestion in varying degrees yielded better percentage comparisons than did plants under normal conditions. An increase of 2 per cent in the total water-content resulted in macroscopically visible signs of water-congestion. Through wilting and subsequent complete water-congestion, the green weight of some leaves may be doubled.

Bacterial Invasion through Stomata. JOHNSON, JAMES. Experiments on the nature of disease resistance require additional evidence of infection without the intervention of microscopic-sized wounds or the propulsion of organisms through stomata by external force. Water-congested leaf tissue results in extreme predisposition to tobacco blackfire and wildfire. Water-soaking by atomizing may result in wounding, and the atomized inoculum may propel organisms through the stomata. Water-congestion produced by the internal water-pressure method, followed by inoculum applied in droplets, eliminates both probabilities. On such water-congested leaves, India ink and bacterial suspensions enter instantaneously, gradually, or not at all, depending upon certain circumstances including stomatal opening. Slight wounding of the water-congested areas results in instant entrance due to capillary tension, and the same force operates through stomata but normally at a slower rate. The size of disease lesions depends chiefly upon the time water-congestion remains in the inoculated area. Bacterial invasion through stomata is less certain than that through wounds, but is sufficiently certain to account for heavy infections frequently found following weather conditions favorable for water-congestion. Under such conditions, for example, dew is sufficient to suspend the parasite and make surface water contact with the water-congestion areas through stomata, permitting invasion to occur by capillary action.

A Spray Boom Adapted for Ground-spraying to Combat Apple Scab. KEITT, G. W. For two seasons spray booms have been designed and tested for increasing speed, economy in cost and manpower, and effectiveness of applying the Elgetol ground treatment. The booms used gave good results. The current model, attached to the rear of the spray rig, is 17 feet long and carries 16 nozzles approximately 18 inches from the ground. It is provided with a shield against low branches, a joint that permits disengagement upon contact with a tree, and a device to avoid excessive variation in distance of the nozzles from the ground. Two men using a rig with a 25-gallon-per-minute pump and 300-gallon tank applied the ground treatment to approximately one acre an hour. In a season of severe scab on McIntosh in unsprayed or inadequately sprayed orchards in 1944, scab development on McIntosh in the experimental ground-sprayed orchard was retarded, mild, and easily controlled. Unsprayed McIntosh trees had 72 per cent of their fruit scabbed at harvest. Various mild programs gave less than 1 per cent of fruit scabbed; *e.g.*, 8 applications of Flotation Sulphur or 3 applications of lime-sulphur before bloom and 5 applications of Flotation or Mike Sulphur or Fermate after bloom. Some blueprints of the boom are available.

Fruit Invasion and Seed Carriage of Tomato Fusarium Wilt. KENDRICK, JAMES B. Vascular invasion by the tomato Fusarium wilt organism (*Fusarium oxysporum* f. *lycopersici*) often extends into the fruit pedicel. Seed removed aseptically from the interior of 265 ripe fruits showing pedicel invasion and planted on potato-dextrose-agar plates showed 20.7 per cent of the fruits to be harboring the wilt organism on the seed. Sixty-

day-old seed extracted from fruits showing a high percentage of *Fusarium* on the seed was treated with a 1–1000 mercuric chloride solution for 5 minutes, washed in sterile water, dried, and planted as whole and as cut seed on agar plates. The whole treated seed remained sterile while the cut seed showed 9.6 per cent and the nontreated seed 100 per cent *Fusarium* growth. Nontreated seed from the same lot planted in sterilized greenhouse soil produced 53.7 per cent wilt-infected plants. Preliminary laboratory trials showed that hot water at 54° C. for 30 minutes did not completely eliminate *Fusarium* from the seed while seed treated with mercuric chloride, 1–2000 for 5 minutes, or New Improved Ceresan, 1–1200 for 5 minutes, showed no *Fusarium* growth on agar plates.

Rhizopus Stem Blight of Tomato. KENDRICK, JAMES B. The canning tomato crop in Central California annually suffers considerable damage from a Rhizopus fruit rot and subsequent blighting of the stem. The fungus enters the early-ripened fruit through growth cracks, worm damage, or other injuries, usually on fruits under heavy foliage where the atmospheric moisture is rather high. After the fruit is entered by the fungus, it becomes a soft watery mass, then loses its watery contents and becomes mummified on the pedicel. In many cases, the fungus penetrates the fruit pedicel, fruit spur, and main branch, causing a blighting of the entire fruit spur and producing elongated dark brown, sunken lesions on the main branch above and below the point of attachment of the fruit spur. Once the fungus enters a main branch of the plant, the leaves show a yellowing and blighting often mistaken for Fusarium wilt. Cultures from internal discolored pedicel and stem tissue have consistently yielded a species of *Rhizopus* similar to *R. nigricans*.

Some Factors That Influence After-ripening of Smut Chlamydospores. KREITLOW, K. W. Chlamydospores of *Ustilago striaeformis* from *Poa pratensis* were after-ripened in host tissue free of contaminating organisms. Spores prepared free of host tissue were after-ripened successfully on filter-paper strips incubated at 35° C. in a moist chamber. Agitating chlamydospores in a Waring Blendor hastened after-ripening and enhanced germinability. Fresh chlamydospores stored at 5° C. for 60 days and then transferred to an incubator at 35° C. required longer to after-ripen than similar spores stored at 25° C. Water in contact with spores was necessary for successful after-ripening. Spores in a saturated atmosphere required longer incubation than when in contact with water, while spores incubated dry failed to become germinable.

Transmission of Virus from X-Diseased Peach Trees to Herbaceous Plants. KUNKEL, L. O. A virus was transmitted from X-diseased peach trees to carrot, parsley, periwinkle, and tomato by means of dodder, *Cuscuta campestris* Yuncker. It was readily transmitted from diseased to healthy plants of each of these species, but all attempts to take it from peach to peach or from these species to peach failed. Since the virus was transmitted from X-diseased trees of four different orchards but could not be obtained from healthy-appearing trees of the same orchards, it seems probable that it was the X-disease virus. In tomato it caused wilting and death; in carrot and parsley it caused chlorosis of young leaves but reddening and yellowing of old leaves; in periwinkle it produced yellowing in both old and young leaves and marked stunting of flowers. All plants into which it was introduced, except tomato, assumed an upright habit of growth and produced an abnormal number of secondary shoots.

The Identity of the Virus Causing Punctate Necrosis and Mottle in Potatoes. LARSON, R. H. Evidence that the virus causing a necrosis and mottle in potatoes is an aberrant strain of the potato latent ring-spot virus has been secured. The virus is transmitted easily by plant extract but all attempts at transmission by means of aphids have failed. The virus remains infective *in vitro* for 28 to 30 days at 20° to 22° C., tolerates a dilution of 1 to 10,000, is inactivated when held at about 66° to 67° C. for 10 minutes, and by drying. Severe symptoms are produced in potatoes at 14° to 16° C. and very mild symptoms when infected plants are held at 24° C. or higher. All Solanaceae tested are readily infected. *Atropa belladonna* L. is a symptomless carrier. Necrotic lesions are produced on the inoculated leaves of *Amaranthus retroflexus* L. and *Digitalis lanata* L.; the virus is not systemic in these hosts. Potato seedling 41956 is immune. Strains of the potato latent mottle virus effectively immunize against the ring-spot virus as measured on such differential hosts as *Nicotiana tabacum* L., *N. glutinosa* and *N. rustica* L. Mixed infections of the ring-spot virus with the veinbanding virus and tobacco mosaic confirmed its affinity with the potato latent ring-spot virus. No evidence was obtained suggesting that more than one virus was concerned.

Further Studies on the Nature and Cause of Purple-top Wilt of Potatoes. LEACH, J. G., AND C. FRANKLIN BISHOP. A 5-year study of purple-top wilt (blue stem) of potatoes in West Virginia has led to the following conclusions: The disease is caused by the aster-yellows virus and is transmitted to potatoes chiefly by viruliferous aster leaf hoppers that have survived the winter as adults. The incubation period of the virus is

longer in potatoes than in asters. Infection must take place not later than early July (in West Virginia) to produce symptoms on late potatoes maturing in early September. Early varieties in West Virginia usually mature before completion of the incubation period, whereas they are severely affected farther north. This probably can be explained by differences in mass movements of leaf hoppers in different regions. All attempts to transfer the virus from potatoes to asters or potatoes, either by grafting or by leaf hoppers, have failed. No satisfactory explanation for these failures can be given. The virus is not perpetuated through tubers from infected plants, although plants from such tubers have low vigor. No practical control measures can be recommended. Low tolerance in certified seed would not aid in control but would tend to insure greater vigor. Roguing to meet a tolerance limit established for this purpose would be justified if tubers were removed.

Inoculation Methods for Testing Blue Grass for Stripe-smut Resistance. LEACH, J. G., AND CONLEY V. LOWTHER. Numerous methods of inoculating blue grass with stripe smut (*Ustilago striaeformis*) have been tested. The conventional methods of seed inoculation, with or without vacuum treatment and with the use of various types of inoculum, have resulted in such low percentages of infection, and the incubation periods have been so long, that they are considered impractical for eliminating susceptible strains. Inoculation of plants near their growing points by means of a hypodermic syringe and with fresh spore suspensions, has given better results. With this method, the incubation period has been as short as 13 days, compared with 6 weeks for seed inoculation. An important advantage of the hypodermic-syringe method is that, without resorting to seed production, clonal selections can be reinoculated over and over, with adequate replications, until resistance or susceptibility is determined definitely.

Growth Rates of Host and Pathogen as Factors Determining the Severity of Pre-emergence Damping-off. LEACH, L. D. Neither the growth rate of the pathogen nor the emergence rate of seedlings adequately explains the relation of temperature to the severity of pre-emergence damping-off. The ratio of the coefficient of velocity of seedling emergence to the growth rate of the pathogen, however, is inversely related to the severity of infection in all combinations of host and pathogen tested. For example, spinach which at low temperature has a relatively greater growth rate than *Pythium ultimum* suffers less pre-emergence damping-off at 4° and 8° C. than at higher temperatures where the ratio of growth rates is reversed. Conversely, watermelons and other high temperature crops show decreasing pre-emergence infection from the same pathogen with each elevation in temperature, a relationship that corresponds to the ratio of growth rates of watermelon and *Pythium ultimum* at these temperatures. A study of ten combinations of hosts and pathogens indicate that this principle has fairly general application. Evidence has also been obtained that seed lots of the same variety differ in susceptibility to Pythium damping-off, a condition apparently correlated with differences in vitality as indicated by emergence rates.

Effectiveness of Seed Treatments Against Surface-borne Ascochyta on Pea Seeds. LEACH, L. D., AND W. C. SNYDER. It has been reported that seed-borne *Ascochyta* is only partially controlled by chemical seed treatments. Failure to obtain complete control may be presumed to be due to internal infection of the seed. Most lots of pea seeds are believed to be relatively free from internally borne infection but in some years many lots carry surface-borne *Ascochyta*. Disease-free pea seed was inoculated by being dipped in a suspension of *A. pinodella*, dried, then treated with various fungicides and planted in sterilized soil. None of the dust treatments completely prevented infection from the surface-borne fungus, but even with the heavy inoculation used, some of the dust treatments gave a high degree of control. At the highest dosages the materials, in descending order of effectiveness, were dichloronaphthoquinone (no. 604), Arasan, Semesan, New Improved Ceresan, Ceresan, Spergon, yellow copper oxide. The dosages of several dusts had a striking effect upon results. Complete elimination of surface-borne *Ascochyta* resulted when seed was dipped in a solution of ethyl mercury phosphate.

Dusting Soybeans for Control of Bacterial Pustule. LEHMAN, S. G. Little information is available on the effect of spraying or dusting soybean plants to control disease. In 1944, a field experiment was set up to determine how much reduction of bacterial pustule (*Xanthomonas phaseoli sojense*) may be expected from dusting the plants with fungicides. The dust preparations used were: (a) 325-mesh sulphur, (b) copper-talc containing 6 per cent metallic copper, and (c) copper-sulphur containing 6 per cent metallic copper. Half the dusted and control plots were inoculated by spraying with *X. phaseoli sojense*. Sulphur dust failed to reduce bacterial pustule. The copper-talc and copper-sulphur dusts were more effective. The variety Tokio had 62 and 90 per cent infected leaves, respectively, in the uninoculated and inoculated control plots and 33 and 51 per cent on correspondent plots dusted with copper-talc. More obvious disease reduction was observed in the number of infections per leaf. Uninoculated and inoculated

undusted plants showed respectively 12 and 60 infections per leaf, but only 3 and 12 infections per leaf were found on correspondent plants dusted with copper-talc. On the more susceptible Herman variety 30 and 100 infections per leaf were found, respectively, on uninoculated and inoculated undusted plants, and only 6 and 15 infections per leaf occurred on correspondent dusted plants. The copper-sulphur dust gave disease reductions closely paralleling those of copper-talc.

An Isoquinolinium Fungicide for Apple Scab Control. LOCKE, S. B. Isothan Q15 (lauryl isoquinolinium bromide), a water-soluble, surface-active, cationic fungicide, was compared with six other materials for apple scab control in an orchard test involving six spray applications on ten single-tree replicates. This toxicant at 1: 5,000 reduced the amount of scab on McIntosh foliage 94.0 per cent below that on unsprayed check trees. Wettable (Camden Paste) sulphur at 1: 80 gave 93.4 per cent control, while Puratized N5D at 1: 20,000 gave 99.6 per cent control. Isothan Q15 and Puratized N5D have therapeutic value as shown by data obtained from tagged leaves. The number of sporulating lesions on McIntosh foliage was reduced 77.2 per cent by one application of Isothan Q15 at 1: 5,000, and 76.9 per cent by one application of Puratized N5D at 1: 20,000. This therapeutic effect is believed to account, in part, for the control obtained. The solubility of Isothan Q15 permits a low minimum effective dosage. Its surface activity increases dispersion of added lead arsenate, reduces arsenical injury, gives a more uniform deposit, and produces better coloring of the fruit.

Deposition of Fungicides and Insecticides on Cherry Foliage. MACK, G. L., J. M. HAMILTON, AND A. W. AVENS. Ineffectiveness of dusts for controlling cherry leaf spot is attributed to inadequate deposition on the under side of the leaves because of lack of moisture. An attachment to a standard duster which allows liquid to be atomized into the dust stream overcomes this difficulty. The deposition of copper materials, sulphur, and lead arsenate applied in equivalent amounts as sprays, dusts, and spray-dusts was compared by chemical analysis of the residues. The copper and arsenic content of the residues from dry dusting was less than half that obtained by spraying. Impregnating dust with oil, 3 per cent by weight, did not increase deposition appreciably. An aqueous 0.1 per cent solution of polyvinyl alcohol applied as a spray-dust doubled the deposit of both copper and arsenic and used only about one-tenth as much liquid as was required for spraying. Leaf-spot control varied directly with the copper content of the residues. Control of leaf spot equal to that secured by spraying was obtained also by the use of a concentrated oil emulsion.

Hop Twine Treatment in Controlling Downy Mildew. MAGIE, R. O. Chemical treatment of twine used to support hop vines partially protected the terminal and lateral buds during May from infection by hop downy mildew (*Pseudoperonospora humuli*). Suspensions and solutions of fungicides in which cotton and binder twines were soaked for 1943–44 field and laboratory tests were Bordeaux, 60–20–100; Tribasic copper sulphate, 6 per cent; Yellow Cuprocide, 4 per cent; Fermate, 4 per cent; U.S.R. No. 604, 2 per cent; No. 604, 1 per cent in chloroform; Spergon, 10 per cent in benzene; saturated water solution of Dithane; Pentachlorphenol, 10 per cent in alcohol; Puratized LN (phenyl mercuri 9, acetoxy 12, octadeconoic acid), 1 per cent, plus Vatsol, 0.2 per cent. Triton X100, 0.25 per cent, was added to water suspensions in 1944 to wet the twine. Puratized LN and U.S.R. No. 604, the more effective materials, permitted one-tenth to one-fifth as much bud infection as was present where the twine was not treated. The copper materials, pentachlorphenol, and Dithane were intermediate; Fermate and Spergon were less effective. The materials fell into the same order of effectiveness when leachings from the weathered twine were tested against sporangia of *P. humuli* on glass. Volatile solvents facilitated the penetration of twine by organic fungicides. Hop vines trained on poles instead of twine were protected similarly by spraying the stacked poles in late winter with the copper materials at 2 per cent copper concentration.

Evaluating Fungicides by Means of Greenhouse Snapdragon Rust. MCCALLAN, S. E. A. A greenhouse method has been developed for evaluating foliage fungicides against a representative rust disease. Plants are sprayed and inoculated under controlled conditions with apparatus developed for tomato diseases (Contrib. Boyce Thompson Inst. 13: 93. 1942). Potted snapdragon plants, variety Cheviot Maid Supreme, are trained to 2 stems and tested when 12 inches tall. Total pustules per 20 leaves, following inoculation with 100,000 spores per cc., are counted 10 days later. Infection occurs readily from 5° to 20° C., with the optimum at 10° to 15° C. The number of pustules is converted to percentage of checks. Straight lines were obtained on logarithmic probability paper, from a dosage series with nine representative commercial fungicides. Empirical probit weights gave highest precision at the LD95 level, as for tomato diseases. One plant per dose suffices, but tests should be repeated. Organic compounds gave the steepest curves and most efficient control, sulphur compounds were intermediate, while copper fungicides with flat slopes were ineffective. Absolute comparisons with laboratory slide-germination

tests showed good agreement with sulphur and organic compounds, but copper fungicides were overrated in the laboratory. Final results are expressed as per cent dosage for 95 per cent control (LD95), or per cent disease at 0.2 per cent spray.

The Use of Venturia inaequalis and Sclerotinia fructicola with Pure Chemical Stimulants in Slide-germination Tests of Fungicides. MILLER, HAROLD J. The recommended procedure of the American Phytopathological Society involves centrifuging of the spore suspension, after which it is necessary to add a stimulant (1) to insure germination above 90 per cent on check slides and (2) to adjust the LD50 to a given concentration of a standard fungicide. Orange juice fulfills both requirements for *Venturia inaequalis* and *Sclerotinia fructicola*. However, different samples of orange juice have caused as much as a fourfold difference in the LD50 value of a dried Bordeaux-mixture deposit. Potassium and sodium citrates at concentrations of 0.001 per cent when used with 0.2 per cent sucrose gave an LD50 in the same range as 0.1 per cent orange juice with less than a two-fold variation and equally good germination on the check slides. There is a linear relationship between concentrations of orange juice, sodium citrate, and potassium citrate and the LD50. Malic and citric acids also have the same properties.

High Calcium vs. High Magnesium Lime in the Preparation of Bordeaux Mixture for Cherry Leaf-spot Control in Wisconsin. MOORE, J. DUAIN. Over a 4-year period, analyses of spray residues on cherry leaves after periods of weathering showed greater retention of copper when Bordeaux was made with high magnesium than with high calcium lime. These differences were obtained with both the 3-spray, 6–8–100 program and the 4-spray, 3–4–100 program, but were somewhat more striking with the 3-spray schedule. The greater difference in the 3-spray program appears to be a consequence of the longer period of weathering in this program wtihout renewal of coverage between the second application and the post-harvest one. Control of cherry leaf spot generally was about the same, whether high calcium or high magnesium lime was used. However, in the years of protracted dry periods with heavy dews, greater defoliation due to spray injury occurred on plots on which Bordeaux made with the high calcium lime was used. When differences in fruit size were obtained between plots sprayed with Bordeaux mixtures made with the two limes, these differences favored the use of high magnesium lime.

Host Range Studies of Necrotic Ring Spot and Yellows of Sour Cherry. MOORE, J. DUAIN, AND G. W. KEITT. Buds from Montmorency trees carrying (1) only necrotic ring spot or (2) cherry yellows and necrotic ring spot were inserted in nursery trees of several varieties of peach and various other species and varieties of *Prunus*. In all cases in which symptoms were expressed, except on *P. cerasus*, the same symptoms were obtained on a given host with either bud source, suggesting the necrotic ring-spot virus as the cause. Budding into ring-spot-free and yellows-free Montmorency from the plants that had been budded a year before from sources (1) and (2) gave only necrotic ring spot if source (1) had been used originally. Both necrotic ring spot and yellows symptoms were obtained on Montmorency trees budded in the spring of 1943 if source (2) had been used originally. Yellows readings on trees budded in the spring of 1944 are not completed. Neither the Abundance nor the Burbank plums used expressed any symptom when budded with either bud source, and neither gave evidence of being a symptomless carrier for either ring spot or yellows. Thus far, all attempts to obtain yellows free from ring spot have failed.

A New Organic Fungicide, 2, 3-dichloro-1, 4-naphthoquinone: Its Value as a Control for Certain Defoliation Diseases of the Tomato. NAGEL, C. M. Results during the 1944 season indicate that 2, 3-dichloro-1, 4-naphthoquinone offers promise in the control of foliage diseases of tomato. Three pathogens which caused epiphytotics during the current season are, in order of importance, *Phytomonas vesicatoria*, *P. punctulans*, and *Septoria lycopersici*. The materials used were: Yellow Cuprocide; DDT, [2, 2-bis (parachlorophenyl) 1, 1, 1-trichloroethane]; Fermate; Copper Hydro 40; 2, 3-dichloro-1, 4-naphthoquinone; He 175; Spergon; and Bordeaux mixture. All materials except Copper Hydro 40 were used as sprays while the first three were used both as sprays and as dusts. Four applications were made between July 14 and August 17. The 2, 3-dichloro-1, 4-naphthoquinone markedly excelled all other materials tested in maintaining plant foliage and caused little or no foliage injury. It was applied at the rate of 1.5 pounds per 100 gallons of water. Yellow Cuprocide ranked second. At the recommended dosages other materials offered little or no protection to foliage. The tomato variety Victor was used in the experiment in a randomized block design with four replications.

The Influence of Temperature on the Susceptibility of Potatoes to Bacterial Soft Rot. NIELSEN, L. W., AND F. A. TODD. Potato tubers exposed to sublethal temperatures become more susceptible to bacterial soft rot. Irish Cobbler tubers were used in laboratory studies on the mechanism associated with this increased susceptibility. Potatoes were stored 10 to 20 days at temperatures from 4° to 40° C. Samples of stored tubers also were heated at 47° C. for 60 minutes as a sublethal treatment. Analyses of juices

from stored and heated tubers showed that increased susceptibility was not correlated with any sugar changes induced by temperature treatments. Changes in cell-membrane permeability were determined from analyses of the sugar contents of diffusates obtained by suspending diced potatoes in distilled water for 2 hours at room temperature. The sugar contents of the diffusates were closely related to the temperature treatments. Exosmosis of sugars progressively increased from potato tissue stored at temperatures above 30° C. The sublethal treatment increased exosmosis from potatoes previously stored below 35° C. The exosmosis of sugars from potatoes stored above 26° C. was correlated closely with bacterial soft rot of early potatoes held four days at the same temperatures. At sublethal temperatures, cell membrane permeability to sugars is a reversible reaction. Possibly it is a factor associated with bacterial soft rot of potatoes exposed to high temperatures during harvest.

Anthracnose of Garden Pea. OU, S. H. Anthracnose (*Colletotrichum pisi* Pat.) occurs commonly on leaves, stems, and pods of the host in Wisconsin pea fields, but is found practically always in association with *Mycosphaerella pinodes* (Berk. and Blox.) Stone. Artificial inoculation results in weak infection of leaves but in no signs of disease on the stems. In nature its conspicuous development appears to be as a secondary parasite following *Mycosphaerella*. All original isolates from naturally infected plants sporulate sparsely on solid media and atypical cylindrical conidia form freely in various liquid media. Colonies on solid media commonly produce sectors in which there is little aerial mycelium and profuse development of falcate conidia typical of those found on the host in nature. The sporulating character usually remains constant during several successive single-spore transfers. Variations in temperature, light, vitamin content of media, reaction of media, carbon source, and nitrogen source have not affected the rate of appearance of sporulating sectors. When the mycelial and sporulating lines are grown together in test-tube cultures the former soon predominates, partly because of its greater growth rate.

A Pythium Tuber Rot and Wilt of Irish Potatoes. PERSON, L. H. A disease of Irish potatoes characterized by a sudden wilting of the plants and a rot of the tubers was found in the spring of 1941 in Louisiana. The outbreak occurred on Katahdin and Triumph varieties during a period of cool weather following a heavy rain. The tuber rot appeared to be distinct from "leak," the tuber being firm and its interior a smoky gray. Isolations from tubers yielded cultures identified as *Pythium debaryanum*. Inoculation of healthy tubers with these isolates produced a rot similar to that found in the field. At the same time inoculations were made with *Phytophthora erythroseptica*, which also occurs in Louisiana. Typical symptoms were obtained, indicating that the rot also was distinct from pink rot. This Pythium rot seems distinct from other rots reported in the United States and resembles in some respects a wilt caused by *Pythium butleri*, reported in Cyprus.

A Rapid Method for Mechanically Transmitting Plant Viruses. RICHARDS, B. LORIN, JR., AND HENRY M. MUNGER. Promising results have been obtained with a high-velocity spray stream in transmitting certain plant viruses. Diluted viruliferous juice, containing 300–400-mesh carborundum, was sprayed on plants through a suction-feed glass atomizer, fabricated of 5-mm. glass tubing, drawn to 0.5–1.0-mm. orifices. Best results were obtained when the atomizer was held 5 to 7 centimeters from the leaves and air pressure above 30 lb. was used. A tank of liquified carbon dioxide proved to be as satisfactory a source of pressure as compressed air and was particularly adaptable for field or greenhouse inoculations. A cut-off assembly (DeVilbiss No. 633) connecting the atomizer to the source of pressure greatly facilitated the spray applications. The method has been used with excellent results to transmit Bean Virus 4 and two strains of Bean Virus 1 to bean plants, and several strains of Cucumber Virus 1 to cucumber. Under direct comparison this spray technique gave percentages of infection as high as, or higher than, did the carborundum-rubbing method. Several thousand bean and cucumber plants were inoculated by spraying with their respective viruses in the field and greenhouse this past season. The results were more satisfactory and the time required was approximately one-fourth of that necessary when the rubbing method was used the previous summer.

Effect of Light Intensity on Infection Types Produced by Races 19, 38, 59, and 59A of Puccinia graminis tritici on Susceptible and Resistant Wheats. RODRIGUEZ V., JOSÉ. High light intensity was conducive to rapid and optimum development of races 19, 38, 59, and 59A on Little Club wheat and other susceptible varieties, with the production of type 4++ uredia. At low light intensity the infection type may be reduced to type 3. All four races produce type 2 uredia on Marquis, but a distinct necrotic band is produced around the uredia under high light intensity, thus limiting the extent of mycelial development. At low light intensity, on the other hand, relative absence of necrosis permits the mycelium to grow more extensively and, although initial sporulation is depressed, secondary and tertiary crops of spores may be produced in diamond-shape bands along the

edges of infected areas, especially if light intensity is increased. This is true also of race 59A on Reliance. Likewise, races 38, 59, and 59A produce type X– infection on Kubanka durum under high light intensity and a somewhat higher infection type under lower intensity, because of the limiting effect of the more pronounced necrosis under the more intense light.

Negative Correlation Between Size of Crown Rust Pustules and Grain Yields in the New Oat Variety, Traveler. ROSEN, H. R. It commonly is assumed that size of pustule indicates degree of susceptibility and consequently is a measure of possible crop losses from cereal rusts. Consistent with this assumption, the oat variety Victoria is considered resistant to common races of crown rust because pustules are usually smaller on this variety than on susceptible varieties. The variety Traveler (Victoria × Custis) was selected because when infected by crown rust it shows a minimum of leaf injury although the pustules are larger than are those on its rust-resistant parent, Victoria. When approximately the same amount of inoculum is applied to Victoria, Traveler, and a susceptible selection from the same cross, the susceptible type has about 10 times as many infections. Killing of leaf tissue is faster in Victoria and Traveler, but the amount of dead or injured tissue is eventually far greater in the susceptible type and apparently proportionate to the number of infections. For five years Traveler has yielded as well as, or better than, other Victoria hybrids with smaller pustules, including sister selections from the same cross. This seemingly suggests that in evaluating susceptibility, size of pustules is not so important as number of infections and amount of leaf tissue injured.

Seedling Blight and Root Rot of Flax in Washington. SCHUSTER, MAX L., and E. J. ANDERSON. Seed coat injury due to threshing, and attack by soil-borne fungi were responsible for poor stands of flax in Washington. Samples of seed varied in the amount of seed with broken coats from 10 to 15 per cent for Zenith and Redwing to 65 to 75 per cent for Viking and Bison. In greenhouse trials, seed threshed by hand produced better stands than did seed threshed by machine at medium cylinder speed. Seed threshed at high cylinder speed produced the poorest stand. Field trials with seed lots threshed by the three methods showed highly significant differences in seedling emergence. *Fusarium oxysporum* Schl. was obtained most frequently in isolations from flax seedlings. Pathogenicity tests showed it to be virulent and the principal pathogen involved in decreased flax stands in Washington. The tests showed also that saprophytic organisms such as *Alternaria* and *Penicillium* may reduce stands. Flax stand was improved by the use of Spergon, New Improved Ceresan, Dubay 1205 FF (50 per cent tetramethyl thiuramdisulfide), and Semesan in greenhouse tests. The first three compounds were equally effective in protecting the seed sown in the field. These treatments produced much greater increases in stands from machine-threshed seed than from hand-threshed seed.

Preliminary Results on the Effectiveness of Elgetol as an Eradicant in Grape Black-rot Control. SHAY, J. RALPH. In a preliminary trial during the 1944 season 1 per cent Elgetol at the rate of 400 gal. per acre was applied to the ground of a one-acre Concord vineyard. The vines were treated with 0.5 per cent Elgetol, 150 gal. per acre. The application was made in the spring after the ascospores of the black-rot fungus had matured but before bud break. An adjoining 2-acre vineyard was used as a control. A measurement of the number of ascospores discharged from mummies collected from the ground in the treated vineyard before and after the Elgetol was applied showed 95.5 per cent suppression of ascospore discharge by the eradicant. Counts of black rot on fruit in late June showed a much reduced level of infection in the eradicant-treated vineyard. Plots in the eradicant-treated vineyard which received no protectant sprays showed about the same amount of infection as plots in the control vineyard which received a full protectant spray program. Fermate gave as satisfactory control in the eradicant-treated vineyard as did Bordeaux 6–8–100 or 3–4–100. No deleterious effect of Elgetol on vigor of the vines could be discerned.

Immunization of Peach Trees to X Disease by Chemotherapy. STODDARD, ERNEST M. Seedling peach trees in pot culture, inoculated with X disease by budding, were injected with chemicals through the cut upper end of the main stem. In 124 trials, *p*-aminobenzenesulphanilamide at various concentrations and under various conditions reduced infection to 21 per cent. At a concentration of 1–2000 injected after inoculation, it completely prevented infection of 45 trees. Check inoculations averaged 85 per cent infection. Maltose or dextrose combined with *p*-aminobenzenesulphanilamide antidoted to a considerable degree its injurious effect on the trees without antidoting its effect on the virus. *p*-Aminobenzoic acid completely antidoted the effect of *p*-aminobenzenesulphanilamide on the virus, just as it antidotes the bactericidal and fungicidal properties. *p*-Toluenesulphanilamide was less effective and less phytotoxic than *p*-aminobenzenesulphanilamide. Hydroquinone, maltose, dextrose, and zinc sulphate reduced infection considerably. It is significant that *p*-aminobenzenesulphanilamide had no therapeutic value in the inactivation of the virus in diseased buds, but that hydroquinone was somewhat effective. With

the exception of zinc sulphate, all the chemicals were more effective when injected two weeks after inoculation than when injected two weeks before inoculation. It is suggested that the inhibitory effects of the chemicals on the virus of X disease is due to artificial immunization.

Experimental Immunization of American Elm from Infection by Verticillium albo-atrum. TEHON, L. R. Immunization of American elm from artificial infection by *Verticillium albo-atrum* appears to have followed injection of staled culture medium and mycelial extract. The fungus was grown on a liquid medium for 10 days and then separated with a Berkefeld candle. This staled medium was used without alteration. The mycelial mat was ground in quartz sand and extracted with water, and the extract was separated with a Berkefeld candle. In August, eight trees 1½ inches in trunk diameter were established in the greenhouse and maintained for two years. In April, by injection, two trees were given staled medium and two trees were given mat extract. In October, November, and January, six trees were given heavy spore injections. By April 1, in the two inoculated check trees there was a general wilting; the fungus was reisolated from them then and in July and in August. The two trees which received injections of staled medium or mat extract, but no spores, exhibited pronounced "anaphylactic" reactions but no wilting. The two trees which received injections of staled medium or mat extract, and spores, did not wilt, and attempts made in April, July, and August to reisolate the fungus from them failed.

Loss Incident to Sprouting among Stored Potatoes Reduced by Hormone Treatments. THOMAS, JOHN E., AND A. J. RIKER. During the warm spring weather any potatoes left in storage have commonly sprouted and soon become worthless. Such sprouting has been prevented by hormone treatments and the selling season has been extended. Among several chemicals tried, the methyl ester of alpha-naphthaleneacetic acid was most successful. It was effective with small variations for the inhibition of sprouting on all varieties tried, *viz.*, Chippewa, Cobbler, Russet Burbank, Russet Rural, Red Warba, and Triumph. Applications of approximately 0.9 gm. per bushel in dilute alcohol or lanolin emulsion sprays, in talc or walnut-shell flour dusts, or on impregnated shredded paper were all effective. Trials were begun in January or February and were run from two to four months at about 70° F. In all cases the checks sprouted and became worthless, many of them rotted. The treated tubers lost some water, but even after several months in warm storage, were still marketable. The treatment became progressively less effective with time. Four to six weeks after treatment Cobbler tubers developed a hard, knobby type of outgrowth, usually in the bud area. In severe cases this growth cracked open.

Seedling Diseases of Sugar Beets in Ohio. TILFORD, PAUL E., AND H. C. YOUNG. Seedling diseases are the principal cause of poor stands of sugar beets in Ohio. *Aphanomyces cochlioides* is responsible for the major loss. Disease caused by *Rhizoctonia* sp. is less common but sometimes very severe. Injury from *Phoma betae* and *Fusarium* sp. occur to a minor degree. The use of high phosphate fertilizers in the row at planting time usually has reduced seedling diseases in the field on soils which have received a liberal application of manure. In greenhouse experiments, row application of either 20 per cent phosphate or complete fertilizer has reduced the Aphanomyces disease but has had little or no effect on diseases caused by the other organisms. Increasing the PO_4 content of culture media from 190 to 9000 ppm. had no retarding effect on growth of *A. cochlioides*. Increasing the concentration of a modified Hoagland solution, to which soybean extract had been added, from 0.8 atmosphere to 2.8 atmospheres had no effect on the growth rate of *A. cochlioides*. When beet seedlings were grown in gravel, inoculated with corn-meal cultures of *Aphanomyces* and irrigated with the same solution minus the soybean extract, seedling loss decreased as the concentration increased from 0.3 atmosphere to 3.78 atmospheres.

Can Tobacco Blue-mold Fungus be Eradicated? VALLEAU, W. D. Although blue mold of tobacco was general in the Georgia-Florida area from 1931 on, it was prevalent throughout the Tennessee-Kentucky tobacco area for the first time in 1937, indicating that this area is not in the direct line of spread of spores from the Georgia area. Since 1937 blue mold has become less and less prevalent until in 1944 only 2 cases were observed or reported in the Tennessee-Kentucky area. This suggests that the blue-mold fungus is not capable of maintaining itself indefinitely north of the Georgia-Florida area. Eradication of overwintering living plants and the use of new plant-bed sites in the Georgia-Florida area should result in the complete eradication of the fungus from the eastern tobacco-growing areas of the United States in a relatively short time.

Progress in Combination of Yellows and Mosaic Resistance with High Ascorbic Acid Content in Cabbage. WALKER, J. C. Wisconsin All Seasons cabbage is heterozygous for the major gene for resistance to yellows (*Fusarium oxysporum f. conglutinans* (Wr.) S. and H.). By selection, homozygous resistant lines of improved type have been isolated.

These lines have been artificially inoculated with the cabbage mosaic viruses (a strain of *turnip virus 1* and a strain of *cauliflower virus 1*). Lines highly resistant to mosaic have been isolated. Within these, lines with 60 mg. or more of ascorbic content per 100 gm. of fresh tissue acid have been secured. With increase of the latter a new strain of Wisconsin All Seasons variety will be available which is homozygous for resistance to yellows, highly resistant to mosaic, and relatively high in ascorbic acid content. (University of Wisconsin and U. S. Dept. of Agriculture.)

A Major Gene for Resistance to Near-wilt in Pea. WALKER, J. C., E. J. DELWICHE, AND W. W. HARE. All commercial varieties of pea resistant to wilt (*Fusarium oxysporum* Schl. f. *pisi* (Linf.) *race 1* S. and H.) are susceptible to near-wilt (*F. oxysporum* Schl. f. *pisi* (Linf.) *race 2* S. and H.). While a degree of tolerance has been attained by selection from certain varieties, such resistance is complex genetically and not readily fixed. In a single plant line from a cross between two varieties, Admiral and Pride, neither of which are tolerant to near-wilt, a major gene for complete resistance has been isolated. This behaves as a dominant to near-wilt susceptibility. The F_3 progenies of a cross with Wisconsin Perfection (resistant to wilt but very susceptible to near-wilt) fit closely the ratio of 1 resistant: 2 segregating: 1 susceptible. Having this line as a breeding parent, the major genes for wilt and near-wilt are being combined with desirable canning and garden varieties.

Seed and Seedling Infection of Barley, Bromus inermis, and Wheat by Strains of Xanthomonas translucens. WALLIN, JACK R. Strains of *Xanthomonas translucens* reduced seedling emergence from artificially infested *Bromus inermis*, barley, and wheat seeds planted on moistened filter paper in Petri dishes or in steamed soil in the greenhouse. Hulling barley had a greater effect on the amount of seedling infection than did the period of soaking in the brome strain of *Xanthomonas translucens*. Wounding the embryos not only lowered germination of the checks, but also gave rise to a larger percentage of infected seedlings in the infested series. Infested hulled barley in comparison with infested unhulled barley planted in steamed soil gave significantly lower emergence and greater numbers of diseased seedlings. The barley, brome, rye, and wheat strains caused seedling infection of barley and wheat, but only the brome strain infected seedlings of *Bromus inermis*. The emergence of the seedlings from wounded and hulled seeds infested with the four pathogens was reduced over the check. The temperature during germination, rather than the temperature at which the seeds were soaked, was the important factor in emergence reduction of barley. Lesions were apparent on seedling coleoptiles within 48 hours. Freehand and paraffin sections showed the bacteria to have infected the coleoptile and the adjacent first foliage leaf. The inner leaves were not invaded at this period.

Host Relation of Xanthomonas translucens. WALLIN, JACK R. A bacterium isolated from the translucent streaks occurring on brome grass leaves was pathogenic on *Bromus inermis*, barley, oats, rye, and wheat causing symptoms on the leaves, leaf sheaths, and culms identical with those caused by one or more strains of *Xanthomonas translucens*. In cross-inoculation studies the brome grass and wheat bacterial strains were pathogenic on Erban oats, *Bromus catharticus, Hordeum vulgare, Secale cereale,* and *Triticum aestivum,* and in addition, the brome strain was pathogenic on Boone oats, *Bromus carinatus, B. erectus,* 7 strains of *B. inermis,* and *B. marginatus.* In a comparison of the pathogenicity of the barley, brome, rye, and wheat strains of *Xanthomonas translucens* inoculated hypodermically into *Bromus inermis,* wheat, Boone and Erban oats, and Peatland and Velvet barley, the brome grass and the barley strains were pathogenic on *Bromus inermis* while the wheat and rye strains were not. All strains were pathogenic on barley, wheat, and Erban oats, while only the barley strain was strongly pathogenic on Boone oats. When the pathogens were sprayed on the leaves, the brome and barley strains were pathogenic on *Bromus inermis* but not on wheat, while the wheat and rye pathogens were pathogenic on barley and wheat. The strains used in these studies do not fit the descriptions of the *formae speciales* for *Xanthomonas translucens.*

A New Dust with Adherence. WATSON, R. D., AND A. A. NIKITIN. One of the chief limiting factors of fungicidal dusts in general is their low adhesiveness. In earlier tests various oils were used but mixtures proved inferior to the standard dust. The new dust mixture is essentially the same as the standard copper 10 per cent – sulphur 90 per cent dust, excepting that 20 per cent clay is added to aid in the dispersion and absorption of 2 per cent oil. The main problem in adding oil was to procure a uniform distribution throughout the dust, which was accomplished by atomizing the oil into the blended dust so that there was no caking even when the oil was used at a concentration higher than that required for improved adhesiveness. It is essential to use a phytonomic (highly sulphonated) oil to avoid copper injury. Solubility tests show that fixed copper fungicides are not affected by the presence of this oil. The distribution of this new dust is more uniform and there is a better coverage on the foliage with less dust than with the standard material. Most important, field and laboratory tests show that it sticks. There

was an evident residue on leaves following more than 3 inches of rain. Tests also indicate that oil tends to minimize the separation of dust into active and inactive ingredients during its application.

A Greenhouse Weathering Technique for Predicting Field Performance of Fungicides. WELLMAN, R. H., AND S. E. A. MCCALLAN. Certain factors in weathering fungicidal deposits on field foliage are: rain, sunlight, wind, leaf growth and excretions, and humidity. Previous greenhouse weathering tests have been essentially rain tests. Significant performance differences were not obtained with Bordeaux and Spergon, even after 5 inches of laboratory rain. The above factors were studied separately and in combination by the "greenhouse tomato" method (Contrib. Boyce Thompson Inst. 13: 93, 1942) and the following technique was developed. The fungicide is sprayed on 4-inch plants, which are returned to the greenhouse. Four times, at 3-day intervals, the plants are subjected to 100 per cent humidity for 16 hours, followed immediately by 0.5 inch of laboratory rain. The plants are then inoculated and later disease readings made. The effect of weathering can be evaluated by comparison with results obtained simultaneously on unweathered plants. Controlling (LD95) late blight on unweathered plants takes approximately: Spergon and Fermate, 0.3 lb. per 100 gallons; Bordeaux mixture, 1.5 lb. (as copper sulphate). After weathering the same control requires: Bordeaux mixture, 4 lb.; Fermate, 8 lb.; while Spergon at 8 lb. gave no appreciable control. This method has been used successfully in selecting compounds for field tests on late blight and apple scab.

The Zinc Salt of Dimethyl Dithiocarbamic Acid (Methasan and Zincate) as a Fungicide on Vegetables. WILSON, J. D. This material has compared favorably with copper, sulphur, and various organic fungicides in the control of the early blights of potato, tomato, and celery, and the anthracnose fruit rot of tomato.

Summary of Results Obtained in a 10-State Experiment on the Control of Tomato Anthracnose. WILSON, J. D., AND H. A. RUNNELS. This experiment included Fermate and Tribasic copper sulphate used alone and in an alternating schedule for the control of anthracnose fruit rot. Pathologists in Maryland, New Jersey, Delaware, Connecticut, Pennsylvania, New York, Ohio, Illinois, Michigan, and South Dakota applied the same formulas in the same schedule to artificially inoculated plots. The disease was scarce in most of the states included in the test, chiefly because of lack of rainfall in late July and August. It occurred in sufficient quantity in many of the inoculated plots, however, to indicate that Fermate may be expected to give a definite reduction in the number of affected fruits.

Puratized N5D, Fermate, and Methasan for the Control of Apple Scab and Bitter Rot. WINTER, H. F., AND H. C. YOUNG. In 1944 orchard tests at Wooster, Puratized N5D (22 per cent), ½ pint per 100 gallons of water, excelled all other treatments in the control of apple scab. Fermate and Methasan gave somewhat less control of scab than the standard Flotation Sulphur schedule. In previous years (1942–1943), however, Fermate was the equal of Flotation Sulphur for scab control. Puratized N5D at the same strength gave perfect control of bitter rot on Grimes in southern Ohio in 1944, whereas Fermate, 2 lb. per 100, and Bordeaux 4–6–100 each reduced the rot to 0.5 per cent. Methasan plus a wetting agent failed to control bitter rot satisfactorily in this experiment. The standard 4–6–100 Bordeaux generally used for bitter-rot control caused excessive foliage and fruit injury. All three organic materials used gave excellent fruit size and finish and caused no perceptible injury on Grimes. Apples sprayed with the Bordeaux averaged 347 to the bushel while those sprayed with the organic materials averaged only 207.

Factors Influencing the Development of Ceratostomella ulmi in Elm Trees. ZENTMYER, GEORGE A., AND PHILLIP P. WALLACE. Nutritional and anatomical differences in elm wood and dosage and age of fungus spores are important factors influencing initiation and development of the Dutch elm disease. Extracts of current season's wood supported much better *in vitro* growth of *C. ulmi* than did extracts of previous season's wood, indicating nutritional variations in different-aged woods. Rapid disease development following early season (May-June) inoculations apparently results from a combination of favorable nutritional substrate and presence of wide rings of large spring vessels. Trees which had been defoliated in June by cankerworms (*Alsophila pometaria* and *Paleacrita vernata)* were much more severely affected than non-defoliated trees when inoculated with *C. ulmi*, possibly because of a prolongation of spring wood formation. Trees which responded vigorously to fertilization with several N sources were not so susceptible to the disease, indicating alterations in nutritional character of wood and sap stream. Severity of disease development increased directly with the number of spores with which trees were inoculated. With an equal number of spores per tree, infection was more severe with multiple inoculation points than for a single point of inoculation. For example, 10 inoculations of 100 spores each were more effective than 1 inoculation of 1000 spores. Old spores were less effective than young spores in producing disease.

EFFECT OF SPRAY INJURY ON PRE-HARVEST DROP OF McINTOSH APPLES[1]

F. H. LEWIS[2]

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Dutton[3] reported in 1932 that pre-harvest drop of Jonathan, Hubbardston, and Baldwin apples varied with the fungicide used in fungicide-lead arsenate sprays. Lime-sulphur caused more pre-harvest drop than Bordeaux mixture. The largest and most consistent differences were obtained when there was a moderate to full crop of fruit. Mills[4] obtained similar differences between lime-sulphur and elemental sulphur on McIntosh in 1935 and 1937.

Lime-sulphur causes considerable leaf injury when used in summer sprays on apple and the amount of pre-harvest drop seems to vary with the extent of leaf injury. Evidence concerning this relationship is presented in this paper.

PROCEDURE

This experiment was conducted in Wayne County, New York, in 1942, with 20-year-old McIntosh apple trees growing in sod. They were in moderate vigor, reasonably uniform in size, and had received uniform treatment for at least the two previous seasons.

Six spray schedules were followed on 7 single-tree plots in randomized blocks for each schedule. Two pre-blossom and 6 post-blossom applications were made at approximately 12-day intervals. Each tree received about 10 gallons of spray in each application; and all spraying was from the top of the sprayer tank with an 8-nozzle boom.

Leaf-injury data were obtained on the leaves of spurs that bloomed in 1942 but failed to set fruit. Forty spurs per tree, taken around the tree at a height of 4 to 6 feet, were used in each instance.

A leaf-injury index was calculated with the object of expressing in one figure both the amount of visible leaf injury on the spur leaves when the counts were made and the amount of leaf fall which had occurred prior to the counts. This was accomplished by the use of classes based on the degree of injury, with the assignment to each class of numerical value; the highest values corresponded with the greatest injury. It was necessary to

[1] An abridgement of one section of a dissertation submitted to the Faculty of the Graduate School of Cornell University, February, 1943, as a major thesis in partial fulfillment of the requirements for the degree Doctor of Philosophy.

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The author wishes to acknowledge his appreciation to Drs. A. B. Burrell, L. M. Massey, and W. D. Mills for their many helpful suggestions and criticisms during this study.

[3] Dutton, W. C. Spray injury studies. 2. Secondary effects of spray injury to apple foliage. Mich. Agr. Exp. Stat. Spec. Bull. 219. 1932.

[4] Unpublished data of Dr. W. D. Mills, Department of Plant Pathology, Cornell University, Ithaca, New York.

assume a base number of leaves before leaf fall began, an assumption which proved to be unjustified. Therefore, an effective-leaf-surface index was calculated as follows: number of healthy leaves $\times 5$, number of leaves with slight marginal necrosis and up to 25 per cent of the margin killed $\times 4$, number of leaves with 25 to 50 per cent of the margin killed $\times 3$, number of leaves with over 50 per cent of the margin killed $\times 2$, and number of yellow leaves $\times 1$. Thus the effective-leaf-surface index is the negative of a leaf-injury index.

The fruits that dropped were picked up at intervals in September and records made of their total number and the number entered by codling-moth larvae. Similar data were obtained on each of two representative crates of picked fruit per tree; and the number of crates of picked fruit was secured. All calculations concerning the fruit were based on these data. All percentages are percentages of the total number of fruits per tree in late August before pre-harvest drop began.

The data were treated statistically by the analysis-of-variance and analysis-of-covariance methods as outlined by Livermore[5] and Snedecor.[6]

RESULTS

The use of lime-sulphur without an insecticide in the pre-blossom and calyx sprays caused a small amount of marginal necrosis and considerable leaf deformity of the type commonly associated with the use of lime-sulphur on immature apple leaves. However, injury did not become severe until Mid-July (Table 1) and was first evident as one or more small dead areas on the leaf margin, often at the leaf tip. These areas enlarged in the more severe cases until a band of dead tissue more or less surrounded a central green area. This central area often became yellow and the leaf fell. Some leaves yellowed and fell without necrosis. The writer believes that the injury after Mid-July was due primarily to arsenic.

The leaf injury varied in severity and time of appearance with the spray treatment. Trees sprayed with lime-sulphur in the cover sprays, with or without lead arsenate and lime, had considerable leaf injury in July. With lime-sulphur alone (Schedule 1), the amount of injury increased in August with little further increase in September. With lime-sulphur and lead arsenate (Schedule 2) or with lime-sulphur, lead arsenate, and lime (Schedule 3), the amount of injury increased in August and September. With flotation-sulphur paste, lead arsenate, and lime (Schedule 4) or with lead arsenate and lime (Schedules 5 and 6), most of the injury occurred in September.

The pre-harvest drop began the last week in August when the majority of the apples were about two inches in diameter. The cumulative percentage fruit drop and other harvest data are in table 2.

Lime-sulphur, lead arsenate, and lime in the cover sprays caused a highly significant increase in pre-harvest drop over lime-sulphur alone; lead arse-

[5] Livermore, J. R. Laboratory exercises in statistical methods of analysis. (Mimeographed.) Cornell University, Department of Plant Breeding. 1939.

[6] Snedecor, G. W. Statistical methods. 422 pp. The Iowa State College Press. 1940.

TABLE 1.—*Leaf injury on McIntosh apple trees by fungicide-insecticide combination sprays*

Spray schedule[a]			July 22 counts				October 1–4 counts				
				No. leaves per spur with part margin dead				No. leaves per spur with part margin dead			
Pre-blossom sprays	Calyx spray	Cover sprays	No. leaves per spur	Up to 25 per cent	Over 25 per cent	Effective-leaf-surface index	No. leaves per spur	Trace to 25 per cent	25 to 50 per cent	Over 50 per cent plus yellowing	Effective-leaf-surface index
1. Flotation sulphur	Flotation sulphur Lead arsenate Lime	Lime-sulphur	8.21	1.87	0.10	38.89	6.65	2.12	0.56	0.29	29.05
2. "	"	Lime-sulphur Lead arsenate	8.11	2.23	0.14	37.89	5.83	2.13	1.13	0.92	21.88
3. "	"	Lime-sulphur Lead arsenate Lime	8.14	2.01	0.20	38.01	5.57	1.93	1.55	1.45	18.40
4. "	"	Flotation sulphur Lead arsenate Lime	8.44	0.21	0.02	42.36	6.75	2.47	0.71	0.29	28.89
5. "	"	Lead arsenate Lime	8.53	0.17	0.04	42.31	6.27	2.65	0.54	0.22	26.84
6. Lime-sulphur	Lime-sulphur	Lead arsenate Lime	8.49	0.60	0.04	41.78	6.31	2.58	0.82	0.39	26.12
Least mean difference for odds of 19:1			0.29	0.58	0.09	1.76	0.41	0.37	0.26	0.33	2.60
99:1			0.39	0.78	0.12	2.37	0.55	0.50	0.36	0.45	3.50

[a] Flotation sulphur paste of the Thylox type obtained from the Rochester Gas and Electric Company, Rochester, New York. Lime-sulphur solution testing 32 to 33° Baumé, NuRexForm acid lead arsenate, and hydrated spray lime obtained from the Sodus, New York, branch of E. I. Du Pont de Nemours and Company. Flotation sulphur paste was used at 8 pounds, lime-sulphur at 2 gallons, and lead arsenate and lime at 3 pounds, in 100 gallons of spray. The order of mixing was the fungicide followed by lime followed by lead arsenate.

TABLE 2.—*Effect of sprays on pre-harvest drop of McIntosh apples*

Spray schedule[a]			Total No. fruits per tree	Cumulative drop as per cent of the total fruits per tree				Drop due to codling moth injury. Per cent total fruits	Picked fruit	
Pre-blossom sprays	Calyx spray	Cover sprays		Sept. 2	Sept. 16	Sept. 22	Sept. 30		Per cent total fruits	Crates of 1.2 bushels
1. Flotation sulphur	Flotation sulphur Lead arsenate Lime	Lime-sulphur	1886	6.65	12.68	16.23	29.27	22.56	48.17	6.8
2. "	"	Lime-sulphur Lead arsenate	1639	5.18	13.37	19.10[b]	31.13	4.92	63.95	8.4
3. "	"	Lime-sulphur Lead arsenate Lime	1844	5.17	16.00	24.78	47.18	5.47	47.35	6.3
4. "	"	Flotation sulphur Lead arsenate Lime	1759	5.08	9.75	13.00[b]	24.24	4.81	70.95	9.3
5. "	"	Lead arsenate Lime	1889	4.26	7.79	9.71	19.54	5.71	74.75	11.0
6. Lime-sulphur	Lime-sulphur	Lead arsenate Lime	1774	5.13	10.37	12.73[b]	21.11	7.74	71.15	10.5
Least mean difference for odds of 19:1 99:1			[c]	.....	3.34 4.49	.....	7.40 9.96	.....	.....	2.5 3.4

[a] See description of materials and concentrations in footnote to table 1.
[b] Sprayed September 17 with "Parmone" at 1 pint to 100 gallons of spray. The active ingredient in "Parmone" is naphthalene acetic acid.
[c] The differences in total number of fruits per tree are not mathematically significant.

nate and lime; and flotation-sulphur paste, lead arsenate, and lime. The data indicate that a heavy hormone application on September 17 was not of sufficient value to overcome the effects of the lime-sulphur spray mixtures. Perhaps the hormone sprays are less effective in reducing pre-harvest drop when the leaves have been previously injured by spray materials.

Since both leaf injury and pre-harvest drop varied with the spray treatment and both were greatest where lime-sulphur was used in the cover sprays, a covariance analysis of the data was used to determine the degree of association between these two variables. Covariance analysis of the effective-leaf-surface index on October 1 and the percentage fruit drop up to September 30 showed a highly significant negative correlation of -0.622. There were highly significant differences between treatments after correction for the "effective leaf surface" on October 1. The data suggest that, in addition to the amount of visible leaf injury on October 1, the fruit drop was affected by the time the injury occurred, the effect of the sprays on leaf efficiency, and the hormone spray applied to 21 of the 42 trees in the experiment.

DISCUSSION

The association between leaf injury and pre-harvest drop shown by the correlation coefficient obtained in this experiment is somewhat obscured by such variables as the use of the hormone spray on part of the plots. It is believed that the excessive dropping of uninjured fruits from these trees can be explained almost entirely on the bases of the amount of visible leaf injury, the time the injury occurred, and the effect of the sprays on leaf efficiency.

The dropping of McIntosh apples before they attain an acceptable degree of maturity and a red color is one of the major faults of the variety. The data presented indicate that the present trend toward the elemental sulphur fungicides will result in less serious losses from pre-harvest fruit drop and perhaps decrease the number of cases where a hormone spray will be needed.

SUMMARY

Under uniform cultural conditions, the amount of visible leaf injury and pre-harvest drop of McIntosh apples varied significantly with the spray treatment. Covariance analysis showed a highly significant correlation between leaf injury and pre-harvest drop. Both were greatest on trees sprayed with lime-sulphur, lead arsenate, and lime in the cover sprays. Flotation sulphur, lead arsenate, and lime in the cover sprays caused less leaf injury in July and August and less pre-harvest fruit drop than lime-sulphur, lead arsenate, and lime.

The thesis is advanced that the excessive dropping of uninjured fruits from these trees can be explained on the bases of the amount of visible leaf injury, the time of injury, and the effect of the sprays on leaf efficiency.

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PHYSIOLOGIC SPECIALIZATION OF PUCCINIA GLUMARUM ERIKSS. AND HENN. IN CHINA[1]

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INTRODUCTION

In a number of wheat growing regions in China, severe losses are sustained annually through stripe rust, a disease caused by *Puccinia glumarum* Erikss. and Henn. It is now almost universally accepted that the most effective and practical method to combat this disease is by breeding or selection of resistant varieties that also are desirable in agronomic characters (2, 4, 12). Before a breeding program to cope with this disease can be effective, however, data should be available on the number of physiologic races of rust present and the reactions of varieties of wheat to those races. In the present paper, the writer brings together the results obtained in determining physiologic races of *Puccinia glumarum* Erikss. and Henn. in Yunnan, China. Data are presented on the reactions of certain highly resistant varieties that are valuable for breeding experiments.

EARLY WORK

In 1894 Eriksson (5) first recognized 5 specialized varieties of *Puccinia glumarum*. They were *Puccinia glumarum tritici* on wheat, *P. glumarum hordei* on barley, *P. glumarum secale* on rye, *P. glumarum elymi* on *Elymus arenarius*, and *P. glumarum agropyri* on *Agropyron repens*. From the later investigations of Hungerford and Owens (10), Gassner and Straib (7, 8, 9) and others (11, 13, 14), it is known that *P. glumarum* comprises many physiologic races and that they are not so narrowly restricted in host range as Eriksson once thought.

The existence of physiologic races in *P. glumarum* was first suggested by Hungerford and Owens in 1923 (10). They found that *Bromus sterilis* was resistant to the rust from *Hordeum jubatum* but susceptible to rust from *Bromus marginatus* and *Elymus glaucus*. It was possible that two strains of *Bromus sterilis* were used or that two races of the rust were involved. Rudorf (12) in his studies on the reactions of 29 wheat varieties to 25 collections of *P. glumarum* from different parts of Europe, found that the races of rust occurring in Europe might be distinct from those in the United States. A year later, in 1930, 4 races were differentiated on 6 varieties of wheat by Allison and Isenbeck (1); and about the same time, 2 distinct

[1] Paper No. 19 from the Division of Plant Pathology, Institute of Agricultural Research, National Tsing Hua University. This investigation was conducted in cooperation with the National Agricultural Research Bureau, Ministry of Agriculture and Forestry. The writer wishes to express his indebtedness to Dr. T. F. Yu for suggestions during the progress of the work and to Prof. F. L. Tai for reading the manuscript.

Phytopathology extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

races in the main wheat growing regions of Western Germany were reported by Gassner and Straib (7). Extensive surveys on physiologic races of this rust have since been made in Europe, United States, and Canada, and at the present time more than 50 races have been identified (3, 11, 13, 14, 15).

MATERIALS AND METHODS

The investigations were made in the greenhouse each year from December to the end of April, in Yunnan, China. The fungus is extremely sensitive to high temperatures, so precautions were taken to keep the temperature below 25° C. by proper shading and ventilation. Seedlings at the two-leaf stage were inoculated after the method of Hungerford and Owens by spreading the fresh uredospores on the upper leaf surface with a scalpel. After inoculation, plants were incubated in moist chambers for 48 hours and then transferred to benches in the greenhouse. The stock cultures, originating from a single sorus, were kept under lamp chimneys covered with a layer of cotton wool to prevent contamination.

The differential hosts used in these experiments included those used by Gassner and Straib,[2] and certain other wheat varieties. Most of the rust collections were made by the writer in Yunnan. Only 6 of the 54 collections studied came from other sources.

The classification of the infection types and host reaction is the one given by Hungerford and Owens (10) with the addition of the immune reaction of Gassner (See 9). Six infection types are thus distinguished: i, 0, 1, and 2 in the resistant class; and 3 and 4 in the susceptible class.

IDENTIFICATION OF PHYSIOLOGIC RACES IN CHINA

In the spring of 1941, 11 collections of the rust, 9 from Yunnan Province and 2 from Szechwan Province, were inoculated on 10 available differential varieties used by Gassner and Straib (See 9), (Bon Fermier, Carsten V, Chinese 166, Heines Kolben, Michigan Amber, Rouge Prolifique Barbu, Strubes Dickkopf, Vilmorin 23, Webster, and Heil's Franken). The results indicated marked differences in the reactions of some hosts to the rust collections. Some of these hosts, however, grew poorly under local field conditions or were of little value as differential hosts for the Chinese rust collections. Ten other varieties, selected from varietal resistance tests in China, were tested as differential hosts, so that a total of 20 varieties were available for further study. Some of the 20 varieties proved not to be differential hosts when inoculated with different collections of the rust; and eventually only 8 of them were chosen as the standard differential hosts: Carsten V, Heines Kolben, Vilmorin 23, Heil's Franken, 9 H 77, Hybrid 128, Carina, and Michigan Amber. Heil's Franken is a barley and 9 H 77 is a common wheat (*Triticum vulgare* Host. var. *ferrugineum* Körn.) from Percival's world collection.

[2] Thanks are due Dr. H. Becker of Halle University, Germany, for sending us seeds of these differential hosts.

It is important to point out here that the variety "Chinese 166," one of the differential hosts used by Gassner and Straib, is well known as immune from or highly resistant to most races of stripe rust in Europe and other countries. It was discarded as a differential host by the writer because it was extremely susceptible to all rust collections in China. It seems probable that the races indigenous to China are different from those in other countries.

Up to the spring of 1943, 9 physiologic races from 43 collections of the rust have been differentiated on the 8 host varieties. The distribution of these 9 races and the infection types they produce on the 8 host varieties are in table 1.

In general, there was a high degree of consistency in the infection type produced by each race, and the types recorded in table 1 are based on 4 or 5

TABLE 1.—*Distribution in China and types of infection produced by 9 physiologic races of Puccinia glumarum on 8 different hosts*

Source and number of rust collections	Infection type on differential hosts								Race designation
	Carsten V	Heines Kolben	Vilmorin 23	Heil's Franken	9 H 77	Hybrid 128	Carina	Michigan Amber	
Kunming—6	4	4	4	i	3–4	3–4	3–4	4	C 1
Chenkung—1	3–4	0	4	i	0	0	2–3	4	C 2
Kunming—18 Paoshan—1 Chenkung—3	0	4	4	i	3	3–4	3	4	C 3
Chengtu—2 Yungchiang—2 Mafong—1	2–3	4	0	i	0	0	4	4	C 4
Tunghai—1 Huaning—1	3	3	3	i	0	2–3	0	4	C 5
Pohsi—2 Hohsi—1 Tunghai—1	3	0	0	i	3	3	0	4	C 6
Pohsi—1[a]	i	i	i	4	i	i	i	4	C 7
Kunming—1	3	3	3	i	0	0	i	4	C 8
Kunming—1	0	0	3	i	0	3	3–4	4	C 9

[a] This rust was collected on barley, whereas all other collections were from wheat.

tests. Since these 9 races are not identical with any of the known ones, they are prefixed with the letter "C" to avoid confusion with the numbered races already reported by others.

Race C 1 is by far the most virulent and it differs from race C 3 in producing type 4 infection on Carsten V. Race C 7, collected from barley in the southern part of Yunnan, produced type 4 infection on Heil's Franken but failed to infect any of the wheat varieties tested except Michigan Amber, a variety highly susceptible to all 9 races. On the other hand, none of the races originating from wheat could infect Heil's Franken. Race C 7 therefore is distinct in its pathogenicity from the rest of the races. Nevertheless,

its ability to infect Michigan Amber makes invalid the strict separation of specialized varieties of *Puccinia glumarum* suggested by Eriksson.

Although the number of collections identified is too small to give precise data on the distribution of physiologic races in Yunnan Province, it is evident that race C 3 is by far the most prevalent in Kunming district, as it occurred in 18 of the 26 collections there. In second place is race C 1, identified in about one-fourth of the collections from Kunming district.

VARIETAL RESISTANCE

During four years, 1487 wheat varieties used in the program for breeding stripe rust and stinking smut resistant varieties for Yunnan were tested

TABLE 2.—*The infection types produced by races C 1 and C 3 of Puccinia glumarum on 30 resistant varieties of wheat*

Accession No.[a]	Variety	Origin	Infection type with C1 and C3
1 B 2	*Triticum monococcum* L. var. *vulgare* Körn.	U.S.S.R.	i–0
30 D 4	*T. dicoccum* Schubl. var. *rufescens* Perciv.	Abyssinia	0
2 E 15	*T. durum* Desf. var. *affine* Körn.	Spain	i–0
14 E 1	" var. *alexandrinum* Körn.	Spain	i–0
2 H 8	*T. vulgare* Host. var. *erythrospermum* Körn.	India	i
2 H 23	" "	Persia	0–2
6 H 3	*T. vulgare* Host. var. *Hostianum* Körn.	Canada	i
6 H 15	" "	Persia	i
8 H 2	" var. *erythrolencon* Körn.	India	0
9 H 23	" var. *ferrugineum* Körn.	Japan	i–0
9 H 66	" "	Portugal	i
9 H 102	" "	Argentina	i–0
9 H 125	" "	U.S.A.	i
9 H 166	" "	Moldavia	i–0
9 H 204A	" "	Poland	i–0
9 H 216	" "	Abyssinia	0–1
14 H 3	" var. *pseudo-barbarossa* Vav.	Persia	i
21 H 15	" var. *lutescens* Körn.	S. Africa	i
21 H 147	" "	England	i–0
21 H 156	" "	Canada	0
21 H 187	" "	England	0
21 H 189	" "	England	0–1
23 H 17	" var. *velutinum* Körn.	unknown	i
24 H 5	" var. *alboretum* Körn.	India	i–0
24 H 6	" "	India	0
24 H 14	" "	Australia	i–0
24 H 62	" "	Tibet, China	0
25 H 121	" var. *nigro-milturum* Körn.	Spain	0
26 H 19	" var. *delfii* Körn.	Iraq	0
8 K 3	*T. compactum* Host. var. *rubriceps* Körn.	China	i–0

[a] Accession number of Percival's collection.

for their reactions to races C 1 and C 3 of *Puccinia glumarum*. In general, none of the Chinese wheat varieties tested was resistant in the seedling stage. Many foreign wheats also were susceptible. There were, however, 30 varieties which were either immune from or highly resistant to the two races. These varieties together with their origin and the infection types produced by stripe rust races C 1 and C 3 are in table 2.

All but 2 of the 30 varieties resistant to races C 1 and C 3 were of foreign origin. It seems to the writer that the introduction of foreign material is indispensable for the breeding of stripe rust resistant varieties of wheat in China.

SUMMARY

Nine physiologic races of *Puccinia glumarum* were identified in 43 collections, most of them from Yunnan Province, China. Race separation was based on differences in infection type on seven varieties of wheat and one variety of barley. Races have been designated as races C 1 to C 9 inclusive.

Of 1487 varieties tested for their reactions to races C 1 and C 3 of *Puccinia glumarum*, 30 were either immune or highly resistant.

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DISTANCE AS A DOSAGE FACTOR IN THE SPREAD OF DUTCH ELM DISEASE

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The dissemination of pathogenic fungi is influenced by many varied factors which are seldom subject to statistical analysis, either singly or in combination. The Dutch elm disease fungus [*Ceratostomella ulmi* (Schwarz) Buisman], however, provides an example of an organism whose dissemination can be studied statistically by establishing plots around isolated infected elms and determining spread outward from these centers.

This fungus is disseminated in the United States primarily by the smaller European elm bark beetle (*Scolytus multistriatus* Marsh.). The principal spread occurs when beetles emerge from dead or dying diseased elms and feed in the twig crotches of nearby healthy trees. Feeding is likely to be concentrated on elms in the vicinity of breeding wood. In the process of feeding, the beetles may inoculate elms with spores picked up from beetle galleries in the diseased elm wood from which they emerged.

This paper presents a study of this local intensification or "local spread" of Dutch elm disease on plots established under conditions of natural infection. Such data have two main applications:

1. The probabilities of infection occurring at varying distances from diseased elms have immediate and important application in local control measures. General statements have been made that local spread of the disease is usually not important beyond distances of from 300 to 500 feet, but no critical examination of this problem has been reported.

2. An opportunity is provided for determining the dose-response relations in dissemination of a pathogenic vascular fungus. Research in various fields of biology has shown that typically the response bears a linear relation to the logarithm of the dosage. This is true whether the response is measured as inhibition of germination of fungus spores when subjected to varying dosages of fungicide, or as disease response in relation to varying dosages of spores, or as response of experimental animals to doses of drugs.

Data by Dimond *et al.* (3) and Heald (6) furnish examples of application of the dose-response principle to plant pathology. Dimond *et al.* investigated extensively the relation between dosage of fungicide and response in incidence of disease or inhibition of spore germination. These factors show a rectilinear relation when plotted on logarithmic probability paper, indicating that the probit of the response varies directly with the logarithm of the dosage. Calculation of data given by Heald demonstrates that the percentage infection (as probits) for stinking smut of wheat increases with the logarithm of the number of spores on the grain.

In the present study, the "response" is measured in terms of numbers of newly infected elms, and the distance of the new infections from the

disease center constitutes the "dosage" factor, as it can be accurately measured while spore dosage cannot in this case. This provides an opportunity for determining the relation between distance and dosage in the inoculum potential complex.

It seems advisable to expand the original definition of inoculum potential as given by Horsfall (7, p. 5) and consider it as "the disease-producing power of the host environment," assuming, of course, that pathogens constitute a part of that environment. Other things being equal, inoculum potential is a function of the dosage of spores. It can be assumed that the dosage of spores normally decreases with distance from the source of inoculum, whether the spores are disseminated by beetles, wind, or some other vector. In spread of Dutch elm disease, therefore, if it is shown that response in percentage infection, as probits, decreases with the logarithm of the distance, then the inverse of distance can be substituted for dosage in the typical dose-response relation. Distance, therefore, could be considered to have an effect inversely equivalent to that of dosage of spores.

METHODS

To obtain pertinent information on the probability of infection by spread of the fungus from diseased elms, isolated, naturally infected trees were selected as centers for three plots in widely separated woodland and roadside areas in Fairfield County, Connecticut. Long distance dispersal of contaminated beetles by wind probably led to infection of these isolated trees. The experimental design for these plots included several requisites: (1) a single naturally diseased tree at the center of each plot, as a source of inoculum; (2) a distribution of healthy elms in the vicinity approaching randomness; (3) separation of the plots from other diseased elms by at least one-half mile; (4) consideration of primary infections only, so that spread from subsequently infected elms in the plot would not confuse the picture; (5) uniformly high level of *Scolytus* beetle infestation on diseased trees at plot centers; and (6) freedom of plots and immediate vicinity from beetle breeding wood.

When first observed in the summer of 1941, each of the naturally infected elms at the center of each plot was severely diseased, and *S. multistriatus* adults were emerging from them. Some emergence undoubtedly occurred also during 1940. By the spring of 1942, all three trees had died from the Dutch elm disease. The diameters (D.B.H.) of these original infected trees were: plot 1, 18 inches; plot 2, 38 inches; and plot 3, 24 inches. Beetles emerged from secondary infections in 1942, so that disease appearance after 1942 could not be attributed to spread from the original diseased trees.

During August and September, 1942, data were taken on subsequent infections resulting from spread of the fungus on these plots. All elms within approximately one-quarter mile of the diseased tree at each plot center were examined for foliar symptoms of disease, and all elms within a 320-foot radius of the original diseased tree were mapped and data were

taken on new infections. Trees suspected of being diseased were sampled and laboratory cultures were made by the usual technique.

RESULTS

The proportion of infected trees on the plots decreased with the distance from the source of inoculum, as might be expected (Fig. 1, Table 1). Inten-

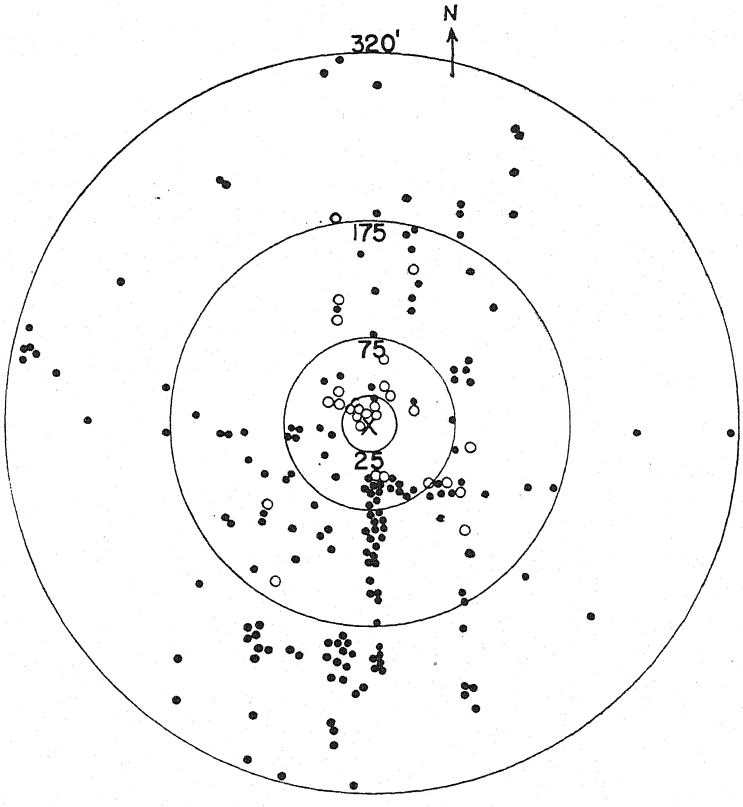


FIG. 1. Diagram showing spread of Dutch elm disease from infection center (X). The diagram combines data from three plots; on each of them, at "X," was an isolated, infected elm.

sification of the disease in the immediate vicinity of the original infections was very marked. Seventy-five per cent of the new infections appeared within 100 feet of the original diseased trees; 40 per cent of the trees within 75 feet of the plot centers became diseased. The maximum distance of spread was 180 feet.

Fourteen diseased elms resulted from the spread of *Ceratostomella ulmi* from the original diseased tree on plot 1 in Norwalk, 8 new infections ap-

TABLE 1.—*Spread of the Dutch elm disease from single trees, naturally infected, on isolated plots in Fairfield County, Connecticut*

Plot	Distance from original diseased elm	Mean distance of all elms within group	Total elms	Elms becoming diseased by 1942	
	Feet	*Feet*	*No.*	*No.*	*Per cent*
1. Norwalk	0.0– 25	13.3	5	4	80.0
	25.1– 75	44.0	12	7	58.3
	75.1–175	124.8	20	3	15.0
	175.1–320	254.0	19	0	0.0
2. New Canaan	0.0– 25	.....	0	.....	.....
	25.1– 75	57.6	17	2	11.8
	75.1–175	108.4	39	5	13.1
	175.1–320	242.8	21	1	4.5
3. Fairfield	0.0– 25	17.4	4	4	100.0
	25.1– 75	57.2	8	1	12.5
	75.1–175	127.2	18	1	5.6
	175.1–320	222.0	32	0	0.0
Totals for all 3 plots	0.0– 25	15.2	9	8	88.9
	25.1– 75	53.2	37	10	27.0
	75.1–175	117.2	77	9	11.7
	175.1–320	236.5	72	1	1.4
			195	28	14.4

peared on plot 2 in New Canaan, and 6 new infections on plot 3 in Fairfield.

The spread data were classified into the following distance groups: 0 to 25, 25.1 to 75, 75.1 to 175, and 175.1 to 320 feet from the plot center. When the mean distances (12.5, 50, 125, and 247.5 ft.) were plotted against the average percentage infection for all three plots on logarithmic probability paper, a linear relation was evident, indicating that the probability of infection decreased with the logarithm of the distance from the disease source.

TABLE 2.—*Summary of statistical analysis of data from Dutch elm disease spread plots*[a]

Variation	n	χ^2	P
Between means of plots	2	2.431	0.29
Between slopes of plots	2	5.401	0.067
About plot curves	5	3.664	0.59
Total	9	11.496	0.24

Combined slope b_c equals -2.504
Regression equation: $Y = 8.865 - 2.504X$

[a] A copy of the statistical data and calculations may be obtained on request.

The data were then analyzed statistically,[1] considering each plot separately. The mean distances of trees within each distance group were calculated, and the data corrected by the use of weighted probits (1). The slopes of the regression lines for each plot and for the three plots combined were calculated, and the significance of the variations between the slopes, between the position of the lines, and about the curves was found by applying a Chi-

[1] The writers are indebted to Dr. C. I. Bliss for assistance with statistical techniques.

square test (1). Significance of the comparisons of variations was judged at a probability level of 1 to 20 (P = .05).

The results of the analysis are shown in table 2. The differences between the positions of the curves (between means of plots) were not significant (P = .29), nor were the differences between the slopes, although the latter approached significance (P = .067). Variations about the plot curves were far from significant. A combined slope (b_c) and regression equation thus may be used to describe the results of the study.

The combined slope "b_c" was calculated by the usual formula and was found to equal -2.504. The regression equation $Y = \bar{y} + b_c(X - \bar{x})$ thus becomes $Y = 8.865 - 2.504X$. The regression curve plotted from this equation is shown in figure 2, with the observed points for percentage infection on each plot at the mean distance for each grouping also shown.

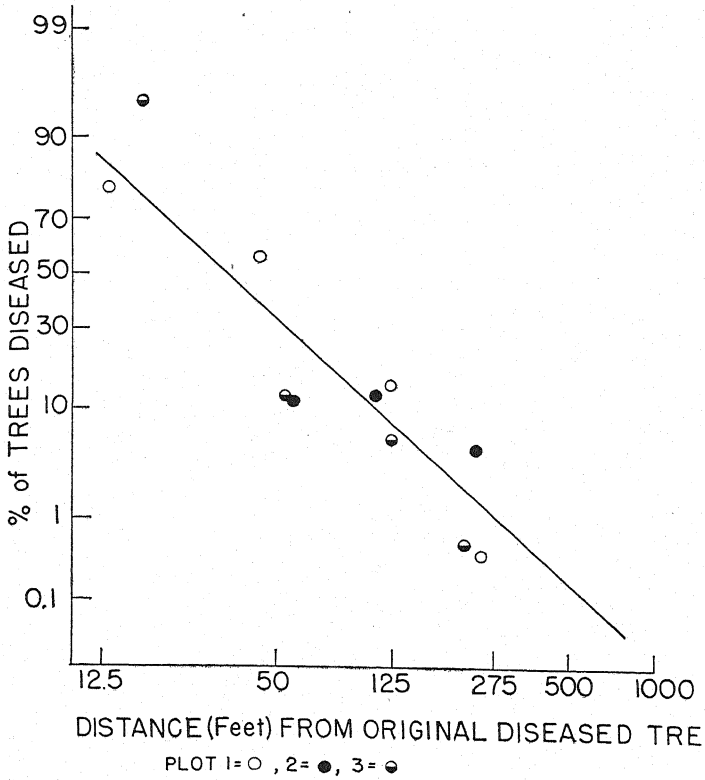


FIG. 2. Regression curve calculated from Dutch elm disease spread data, plotted on logarithmic probability scale. Points along the calculated line are observed infection percentages for the mean of each distance group on each of the three plots.

From the regression line (Fig. 2) it is apparent that when all probabilities are combined the chances of infection for an elm within 25 feet of the original diseased tree were about 6 in 10. At 50 feet the chances were about 3.5 in 10; at 100 feet, 1 in 10; at 300 feet, 1 in 100. The accuracy of extrapolated values is particularly enhanced by the precision of the calculated

regression line, and it can be seen from the graph that the probabilities of infection at 500 feet were about 1 in 500, and at 1,000 feet they were approximately 1 in 10,000.

Inspection of the composite map (Fig. 1) revealed a considerably larger percentage of new infections in the north half of the plots than in the south half. Of 65 elms in the north half, 18 or 27.7 per cent became infected, as contrasted to 10 infections in 130 elms (7.7 per cent) in the south half. When the data were adjusted for the difference in initial population and were analyzed statistically by a Chi-square test, the difference between the percentage of infections in the north and south halves of the plots was highly significant (χ^2 between means equals 5.526, n = 1, P = .01). The slopes are practically parallel (χ^2 between slopes = .011, n = 1, P = .92).

Calculation of the relative level of infection (antilog M) (9) demonstrated that the spread of infection to the north was twice as great as to the south of the plot centers (antilog $M = 2.0 \pm .7$, $M \pm s_M = .301 \pm .15597$). For example, where the distance for 50 per cent infection on the north was 50 feet, that on the south was 25 feet. The χ^2 between means and the comparison of M with its error reveal that the difference between north and south is just significant according to statistical measures.

DISCUSSION

This evidence that the probability of infection in Dutch elm disease decreases linearly with the logarithm of the distance from the source of inoculum provides further data on the relation between dosage and response in biology. The premise that the distance from a source of inoculum might be considered to bear an inverse linear relation to dosage of spores is substantiated. Since the mechanism of penetration is via beetle feeding punctures, the number of punctures would be expected to decrease also as the distance increases logarithmically. When data presented by Wolfenbarger and Jones (10) are plotted on logarithmic probability paper this is the case. Essentially the main factor determining the response, however, is the effective spore dosage reaching the tree, regardless of the vector.

If distance acts similarly to dosage, this relation should appear in other work. Re-calculation of data presented by McCallan (8) shows that the percentage germination (as probits) of *Sclerotinia americana* conidia at varying distances from a drop of Bordeaux mixture on a glass slide increased with the logarithm of the distance. Also, when data of Bonde and Schultz (2) were plotted on logarithmic probability paper, the percentage (as probits) of potato late blight in a field decreased with the logarithm of the distance from infected refuse piles. Hence the same laws are operating whether the distance factor is concerned with the amount of Dutch elm disease, beetle feeding punctures on elm, the effectiveness of Bordeaux mixture, or spread of potato late blight. Distance in all of these cases follows the same dose-response principle as do dosages of spores in producing disease, or of fungicides in preventing disease.

Frampton, Linn, and Hansing (5) presented data on the spread of the yellow-dwarf virus into potato fields and of the aster-yellows virus into endive beds. Data from some of these fields, when replotted on logarithmic probability paper, follow the log-probit dose response relation. Data from other fields do not follow this relation, however; the types of curves obtained with these indicate that complicating factors such as multiple sources of inoculum may be affecting the spread pattern.

These data also furnish information of significance in control of a specific disease. Elm owners wish to know the probabilities for infection in a group of elms at a given distance from a source of inoculum such as a diseased tree or a pile of infested wood. Control officials are concerned from the point of view of the distance (width of local control zone) required for a low level of infection. The elm owner is interested in the probabilities of infection at a given distance. The control official wishes to know the distance for any given probability of infection. Either can be obtained by interpolation on a regression line, as in figure 2. Both are determined by two characteristics of the regression line: position and slope.

The position of the line should be governed by: (1) the amount of fungus inoculum present, (2) number of beetles, (3) number of elm trees and the randomness of their distribution. With an increase in the amount of fungus or number of beetles, or a decrease in the number of elms, the regression curve should shift to the right.[2] Under these conditions the width of the protective zone should be widened and the probability of infection at any given point would increase. Under opposite conditions the regression line should shift to the left and the probability of infection at any distance would decrease.

The slope of the regression line will presumably be altered by various site, topographical, and weather factors, and by extraneous factors such as the presence of devitalized or dying elm wood which would attract beetles for breeding sufficiently to change expected infection percentages. If the elm distribution does not approach randomness, these generalizations will undoubtedly be confused.

Local spread is not restricted to the relatively short distances found here by limitation in flight or dispersal of the vector. The distance of occasional, chance dispersals of *Scolytus multistriatus* is known to be more than two miles and may in some instances extend to greater distances.

Wind is apparently a factor in the long distance dispersal of *S. multistriatus* adults (4), and hence in the spread of Dutch elm disease. The current data indicate that wind also influences local spread. The difference between the relative amount of infection in the north and south halves of the combined plots was significant; wind was probably the main factor responsible for this difference. The prevailing winds are from the south in the plot localities during months of beetle emergence. These results

[2] Data of Wolfenbarger and Jones (10) tend to confirm this. Plotting their data on logarithmic probability paper showed displacement of the regression line to the right when five times as many beetles were present, but the slope of the line was not affected.

demonstrate the importance of exposure and topography in determining the width of local control zones.

The data for these three plots and a discussion of the factors which affect local spread of Dutch elm disease provide useful information for estimating protective barrier zones in local control. It should be recognized that considerable variation in probabilities of infection can be expected with varying local conditions and that these calculated probabilities apply only to the average conditions on these 3 plots.

SUMMARY

Statistical analysis of data from 3 plots to study spread of the Dutch elm disease from large, naturally infected elms showed that the probability that a tree will become infected decreased directly with the logarithm of the distance from the disease source. The spread of this pathogenic fungus (*Ceratostomella ulmi*) therefore is in agreement with the dose-response relation that occurs in many fields of science. Since a rectilinear relation exists between the logarithm of the distance and the percentage infection (as probits), distance from a source of inoculum has an effect inversely equivalent to that of dosage of spores.

By calculating regression equations for each plot and comparing the variations by a Chi-square test, the data were found homogeneous; the 3 equations could be combined to give one regression line to describe the observations. This calculated line is useful in determining probabilities of infection of trees at varying distances from the disease source, and thus has significance in local control measures.

Intensification of disease was very marked in the immediate vicinity of the original infected elms. Seventy-five per cent of the new infections on the 3 plots were within 100 feet of the original diseased trees; 40 per cent of the elms within 75 feet of the disease source became infected.

The distance of dissemination of the fungus to the north of the plot centers was approximately twice that to the south; the distance for 50 per cent infection on the north was 50 feet, on the south, 25 feet. Statistically, the difference in amount of infection for the two halves was at the margin of significance. Prevailing south winds during beetle emergence apparently caused this divergence.

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STANDARD AND NEW FUNGICIDES FOR THE CONTROL OF COVERED KERNEL SMUT OF SORGHUM AND THEIR EFFECT ON STAND[1]

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The sorghum grower in the Southern Plains area of the United States is constantly confronted with the problem of obtaining satisfactory stands of plants free from covered kernel smut. Experiments at the Kansas Agricultural Experiment Station (1, 4) were the first to demonstrate that chemical dust treatments were more satisfactory than wet treatments for the control of covered kernel smut. Treatment of sorghum seed soon became an adopted farm practice on a large acreage in Kansas and other sorghum-producing areas. Copper carbonate and New Improved Ceresan, the fungicides most often recommended, have satisfactorily controlled smut and are of value in promoting good stands, but they have some limitations.

When unsatisfactory environmental conditions prevail between the date of planting untreated sorghum seed and emergence of seedlings, the percentage stand of plants is reduced considerably. Fearing poor stands, the grower generally sows more sorghum seed than is desirable. This may give a good stand under unfavorable environmental conditions; on the other hand, when environmental conditions are favorable, too heavy a stand is obtained, which is detrimental under conditions of average or below average precipitation. Fungicides that will stabilize the stand when a recommended rate of seed is planted are desirable, especially when they also control smut. The use of such fungicides would enable the grower to conserve seed and to determine more accurately the rate of seeding based on laboratory seed germination tests.

An experiment was designed to learn what chemical dusts achieve satisfactory control of covered kernel smut, promote better stands than the dust fungicides commonly used, and improve the vigor of the seedlings, especially when in cool, moist soils. Corona Coppercarb and New Improved Ceresan (5), with the new fungicides Arasan, Spergon, and DuBay 1452–C, micronized free-flowing sulphur, and micronized wettable sulphur were used. The sulphur dusts were of immediate importance because of the wartime critical shortage of copper.

Pink kafir sorghum seed, with a laboratory germination of 93 per cent, was inoculated with covered kernel smut at the rate of 1 gram of spores to 200 grams of seed. On April 20, 1943, separate portions were treated with the inorganic fungicides, micronized free-flowing sulphur, micronized wettable sulphur, and Corona Coppercarb, each at the rate of 3 oz. of the chemical dust per bushel of seed. Similar portions of seed were treated with

[1] Contribution No. 457 from the Department of Botany, Kansas Agricultural Experiment Station.

the "nonvolatile" organic fungicides Arasan and Spergon at 2 and 3 oz. per bushel, respectively, and with the volatile organic fungicides New Improved Ceresan and DuBay 1452–C (7.7 per cent ethyl mercury-p-toluene sulfonanilide), each at ½ oz. per bushel.

Two days later four 20-gram lots of seed from the untreated sack and from each of the treated sacks were planted in the field in randomized 75-foot rows. Each treatment was replicated four times. The seed was planted at an earlier date than sorghum seed is usually planted at Manhattan to increase the probability of there being environmental conditions unfavorable for seed germination between planting and emergence of the seedlings. This earlier date of planting would generally give greater comparative differences in stand of plants between treatments than would later

TABLE 1.—*Effect of seed treatment on the stand of sorghum plants and on the control of covered kernel smut.*[a] *Manhattan, Kansas, 1943*

Seed treatment	Rate per bu.	Av. emergence of plants		Final stand of plants		
				Mature	Smutted	
	Oz.	*No.*	*Per cent*	*No.*	*No.*	*Per cent*[b]
No treatment	.....	360	34	457	251	56.8
Micronized free-flowing sulphur	3	366	34	451	4	0.9
Micronized wettable sulphur	3	367	35	458	7	1.3
Corona Coppercarb	3	609	57	569	24	4.2
Arasan	2	713	67	560	2	0.4
Spergon	3	749	71	561	2	0.4
New Improved Ceresan	½	657	62	556	0	0.0
DuBay 1452–C[c]	½	648	61	533	0	0.0
Difference necessary for significance:						
5 per cent level	.....	85	.....	.....	.....	5.0
1 per cent level	.....	116	.....	.....	.....	6.8
0.1 per cent level	.....	156	.....	.....	.....	9.2

[a] Seed treated April 20 and planted April 22; emergence notes taken May 26 to 27; plants thinned May 28 to 29; smut notes taken September 1.
[b] Average of percentages.
[c] 7.7 per cent ethyl mercury-p-toluene sulfonanilide.

plantings. Stand counts were taken five weeks later when the plants were several inches tall. The data were analyzed statistically by analysis of variance.

There was no significant difference in stand of plants from seed which was untreated (34 per cent) and that which was treated with either of the sulphur fungicides (34 per cent and 35 per cent) (Table 1). There was no apparent beneficial or detrimental effect due to the sulphur seed treatments. This substantiates the earlier work of the writers and other investigators (1, 2, 3, 4, 6) in which similar stands of plants were obtained from untreated seed and seed treated with sulphur.

The stand of plants for Corona Coppercarb was 57 per cent; for Arasan, 67 per cent; for Spergon, 71 per cent; for New Improved Ceresan, 62 per cent; and for DuBay 1452–C, 61 per cent (Table 1). The increase in stand

from seed treated with either Corona Coppercarb or any of the organic fungicides over untreated seed was highly significant. In this experiment the stands of plants for the treatments Spergon and Arasan were the highest, each being significantly higher than that for Corona Coppercarb, and together being significantly higher than for New Improved Ceresan and DuBay 1452–C. Recently better stands from seed treated with Spergon and Thiosan (Arasan) than from seed treated with New Improved Ceresan have been obtained elsewhere (2). DuBay 1452–C was about equal to New Improved Ceresan in increasing the stand of sorghum plants in the experiments at Manhattan (Table 1).

After the stand counts were taken the plants were thinned to approximately 7-inch intervals. When the plants headed, notes were taken on the total stand and on the number of smutted plants. Percentages were calculated and the data were analyzed statistically by analysis of variance. The smutted plants for the untreated checks averaged 56.8 per cent, for Corona Coppercarb 4.2 per cent, and for the other treatments from none to 1.3 per cent (Table 1). The difference between the percentages of smutted plants for the untreated check as compared with any of the fungicide treatments was highly significant. There was no significant difference in the percentage of smutted plants for any of the fungicide treatments.

Summing up the results of this experiment, the fungicides Spergon, Arasan, New Improved Ceresan, DuBay 1452–C, and Corona Coppercarb were satisfactory in increasing the stand of sorghum plants. The two sulphur fungicides had neither an apparent beneficial nor a detrimental effect on the stand of sorghum. All of the fungicides used effectively controlled covered kernel smut. Thus, several efficient, economical, and practical seed disinfectants are available other than those in which copper and mercury are the active ingredients. This matter is important because of war conditions.

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STRAINS OF PRUNE DWARF[1]

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(Accepted for publication June 24, 1944)

In recent years prune dwarf, attributable to Prunus virus 6 (13) or *Nanus pruni* H. (10), has been observed at widely separated points in the United States and Canada (9). First discovered in New York State in 1930 and reported in 1936 by Thomas and Hildebrand (14), it has since been observed in the Niagara Peninsula of Ontario in 1937 (1) and in the Penticton district of British Columbia in 1938 (12). Hitherto prune dwarf has been found in a recognizable form in nature, only on Italian prune (12, 14), and on Italian prune topworked on Damson (1, 8). Hildebrand (8) not only has demonstrated that Damson could carry the virus without showing symptoms but also has transmitted prune dwarf by budding to several prune type plums, to Lombard plum, and to peach, but not to cherry. The present article, by comparing symptom expression on a series of common hosts, submits evidence that two viruses from naturally infected sweet cherry and two from plum or prune are in reality four strains of prune dwarf virus.

SOURCES

The disease was first discovered in Ontario in an orchard near Grimsby, where Damson had been topworked to prune. Preliminary indexing on peach seedlings gave some not very convincing signs of transmission. Strain 1 was obtained in 1939 from Italian prune, originally from the same orchard, that had been grafted on Damson in a neighboring orchard. Grand Duke plums on Damson in this orchard were apparently healthy.

Strain 2 came from the Beamsville district in 1939, from an unknown variety of blue plum with striking symptoms of prune dwarf after being topworked on Damson. Italian prune on Damson at the same location was also typically affected in some instances but not in others, indicating that some of the Damsons were healthy.

The two viruses procured from sweet cherry are designated, for convenience, as strains 3 and 4 of prune dwarf, but for the time being they should be regarded as causing separate diseases. Strain 3, which causes Elkhorn ring spot (9), was found in a garden near St. Catharines in 1940 on a tree of the Elkhorn variety, some leaves of which were marked with small ring spots often with necrotic centers, and with larger rings and blotches (Fig. 3, J), while the fruit had small sunken areas and internal brown spots. The leaf symptoms in the illustration are somewhat exaggerated, being photographed with transmitted light. The tree was symptomless in 1941, but, as there was no way of knowing how long it had had the disease, it was impossible to decide whether the striking symptoms of 1940 were due to the cool,

[1] Contribution No. 777 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

wet season or to the initial shock of infection. Both the shock effect and the influence of temperature on symptom expression have been noticed in connection with other viroses on Prunus hosts (1, 5, 6, 11).

Strain 4, responsible for Eagle mottle (9), was picked up in 1940 near Niagara Falls on sweet cherry, variety Black Eagle. The owner's cause of complaint was the tree's unfruitfulness for several seasons. The only other symptom observable in June, 1940, was an inconspicuous foliage mottle.

A fifth strain was obtained from Damson trees in an orchard near Winona in 1940 but has not been used in these experiments because preliminary trials indicated close relationship with strain 1.

EXPERIMENTAL HOST RANGE

In the experiment, 14 host varieties, together with seedlings, were used, namely: *Prunus domestica* var. Italian prune, German prune, Lombard, Reine Claude, and Grand Duke; *P. salicina* var. Abundance; *P. persica* var. Elberta, Rochester, and seedlings; *P. cerasus* var. Montmorency; *P. avium* var. Napoleon, Black Tartarian, Black Eagle, and Elkhorn; *P. armeniaca* var. Niagara; *P. cerasifera*, Myrobalan seedlings; and *P. mahaleb*, seedlings.

METHODS

The complete series of inoculations was essentially the same for each of the four strains and was developed over a period of three years as illustrated in figure 1. In 1940 and 1941, triplicate series of seedling peaches of the current year's growth were double-budded, first with a bud carrying the virus strain under investigation and then with a bud of the desired "healthy" variety. In 1942, direct inoculation on 2-year nursery stock, in duplicate, was employed for some varieties and double-budding, in triplicate, for others (Fig. 1). Throughout the double-budding experiments, each of the "healthy" varieties was of clonal origin indexed as required. The results of indexing are not shown in the chart (Fig. 1) but the clons were healthy except as noted. The nursery stock varieties were obtained direct from a local nursery and were thus of unknown history and without definite proof of health. Because of the possibility of the occurrence of masked viroses in certain stone fruits, the use of unindexed nursery stock is not entirely satisfactory and the lack of the necessary seedlings unfortunately made it impossible to index the 196 individuals in the plantation. However, no indications of virus disease were apparent in the nursery stock during the summer of 1942. The plums and peaches in this block showed no signs of prune dwarf in 1943 except where inoculated with one or other of the four strains and so could be assumed to have been healthy so far as that disease was concerned. Half the cherries, both in the four prune-dwarf series and in series inoculated with other viruses, were indexed in 1943 on peach and Italian prune (Fig. 1) to verify transmission and to test the purity of the transfer.

The double-budding technique was in some respects better than the inoculation of larger trees because, besides facilitating indexing and the use of

clonal stock, it tended to give an earlier expression of symptoms. There was, however, some difficulty in getting one or two of the plum varieties to grow on peach stock, while the cherries failed to grow on peach and were somewhat

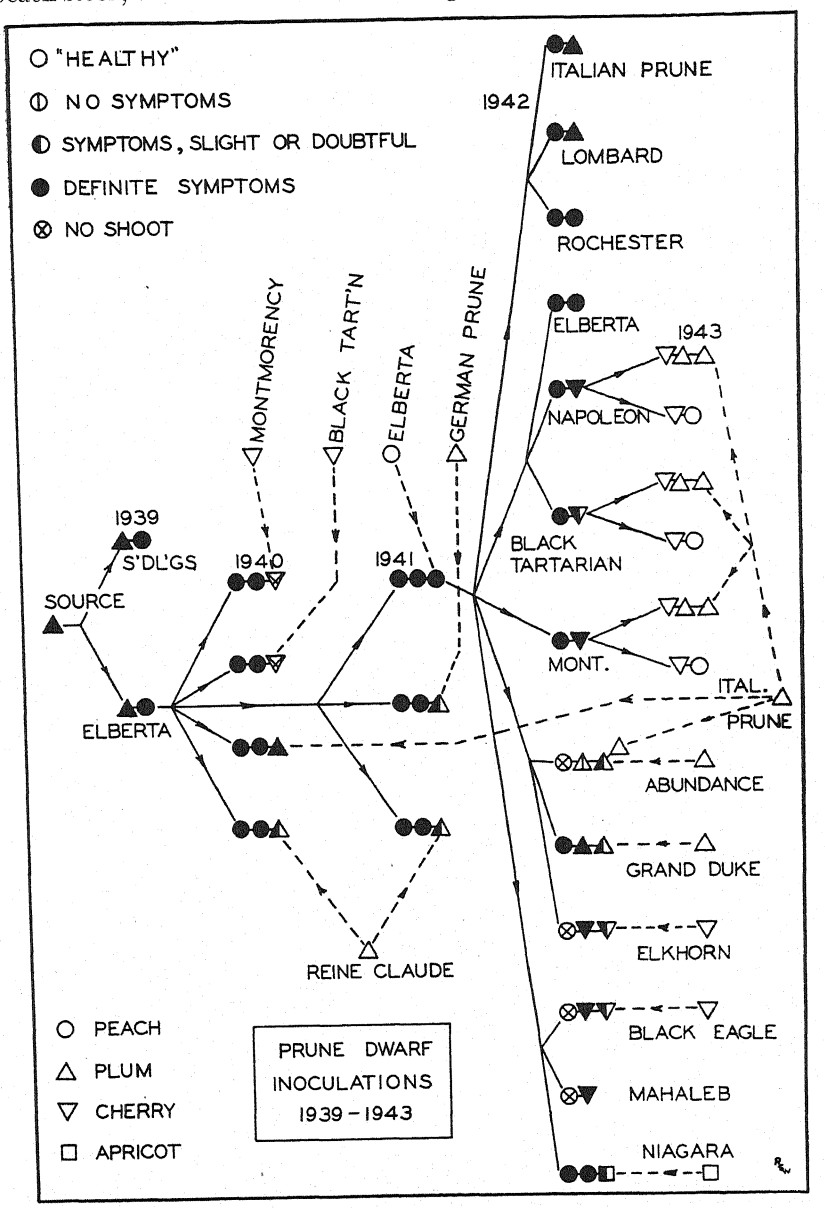


FIG. 1. Typical inoculation scheme tracing the transmission of a strain of prune dwarf from the original source through sub-inoculations and showing the varieties tested and the development of clons in "the double-budding" experiments. All sets of inoculations in triplicate except on nursery stock in 1942 which were in duplicate.

uncertain even on Mahaleb. The failure of inserted diseased buds to produce shoots did not prevent transmission of the virus, even where the whole

TABLE 1.—*Synopsis of symptom expression*[a] *of prune dwarf virus strains on various stone fruit varieties*

Host	Strain 1	Strain 2	Strain 3	Strain 4
Italian prune	A, B, C, D ±	A, B +, C +, D ±	A, B, C, D ±	A, B, C, D ±
German prune	D −/=, R	D −	D −/=, R	D −/=, R
Lombard plum	A ?, B, C, D −	A ?, B, C +, D, E +	A ?, B, C, D −	A ?, B, C, D −
Reine Claude plum	A −, C −, R	A, C, D	O	A −, C −, R
Grand Duke plum	D −/=	D	.....	.....
Abundance plum	D ?/=	D −	D ? /=	D ? =
Elberta peach	A −, C, D =, E, R ±	A, C +, D, E, F ±	A −, C −, D −/=, E −, F ±, R ±	A −, C +, D =, E, F ±
Rochester peach	A −, C, D =, E, R ±	A, C +, D, E	A ?, C −, D −/=, E −, F ±, R ±	A −, C +, D =, E, F ±
Seedling peach	A −, C, D =, E, F ±, R ±	A, C +, D, E, F ±	A ±, C −, D −/=, E −, F ±, R ±	A −, C +, D =, E, F ±
Montmorency cherry	D −	C ?, D	D − /=	A −, D
Napoleon cherry	D −	C −, D	A ?, C −, D −/±	C −, D
Bl. Tart 'n cherry	D −/±	D −	O	D
Black Eagle cherry	D ?	D −	D ?	D ?
Elkhorn cherry	O ?	D ?	D ?	.....
Niagara apricot	A, C −	A, C, D ± ?	A −, C ±, D ?	A −, C
Myrobalan sdlgs.	O ?	C ±, D ±	O ?	O ?
Mahaleb sdlgs.	D ±	D	D ±	D ±

[a] Key:— O: No symptoms; A: Delayed foliation;
B: Strapping and rugosity of leaves (characteristic on prune);
C: Dwarfing (reduction in number and length of internodes);
D: Markings on leaves; E: Wavy leaf margins;
F: Superficial bark necrosis; R: Recovery.
=: Transient; −: faint or slight; +: pronounced or severe; ?: doubtful; ± may or may not occur.

graft-piece died during the winter. As all budding was done in August or early September, the phrase "the first season" refers not to the year of budding but to the next following.

SYMPTOMATOLOGY

The effects of the four strains on the various hosts of the range are compared in synoptic form in table 1.

On Italian prune.—All four strains produced the characteristic symptoms of prune dwarf. One of the earliest indications of transmission was a delay in the opening of buds. Leaves, as they developed, became strap-like, narrow, somewhat thickened, and sometimes emarginate, with roughened or pebbled surfaces and frequently a fine mosaic mottle (Fig. 2, A). Affected leaves and shoots were smaller than normal. Symptoms appeared in the first season in the double-budding experiments but not on the nursery stock except when the inoculated branch was cut back and then only near the point of inoculation. Some differences in severity of reaction were noted, strains 2 and 4 being more severe than strains 1 and 3. For example, prune buds in the 1940 experiment with strain 2 put out in the spring of 1941 severely affected and dwarfed shoots which died during the summer. Prune shoots inoculated at the same time with strain 3 have survived for at least three seasons though showing quite striking symptoms annually. The symptoms of the two cherry strains, 3 and 4, on Italian prune are shown in figure 2, E and G.

On German prune.—With strains 1, 3, and 4, some transient mild mottle, followed by whitish or chlorotic areas on some leaves, was observed in the first season. No symptoms were evident in the second season. With strain 2, however, the mottle was accompanied by a slight distortion and blistering of early leaves, while faint patterns mostly of the oak-leaf type appeared in June (Fig. 3, K). The latter symptoms occurred again in the second season.

On Lombard plum.—On nursery stock, all strains caused slight chlorotic flecking and spotting early in the season and dwarfing of shoots, localized near the inserted diseased buds. Where the inoculated branch was cut back to the bud, the dwarf shoots bore leaves very much like those of affected Italian prune (Fig. 2, D). Strain 2 was more nearly systemic in its effects and in addition to the localized symptoms caused a variety of chlorotic patterns such as lines, rings (Fig. 2, C) and marginal V-shaped blotches, and some necrotic spotting, while many leaves were distorted by twisting, asymmetry, and pronounced waviness of the laminae, frequently associated with the chlorotic areas (Fig. 2, B). Some shortening of internodes tended to give the new growth a semi-rosetted bunchy appearance in the late summer.

On Reine Claude plum.—In the 1940 and 1941 experiments with strains 1 and 4, Reine Claude showed no symptoms beyond a slight delay in foliation and slight dwarfing in the first season only. Strain 3 had no apparent effect. With strain 2, however, growth has been stunted and oak-leaf and coarse line patterns of varying intensity have appeared in June and July for at least three seasons.

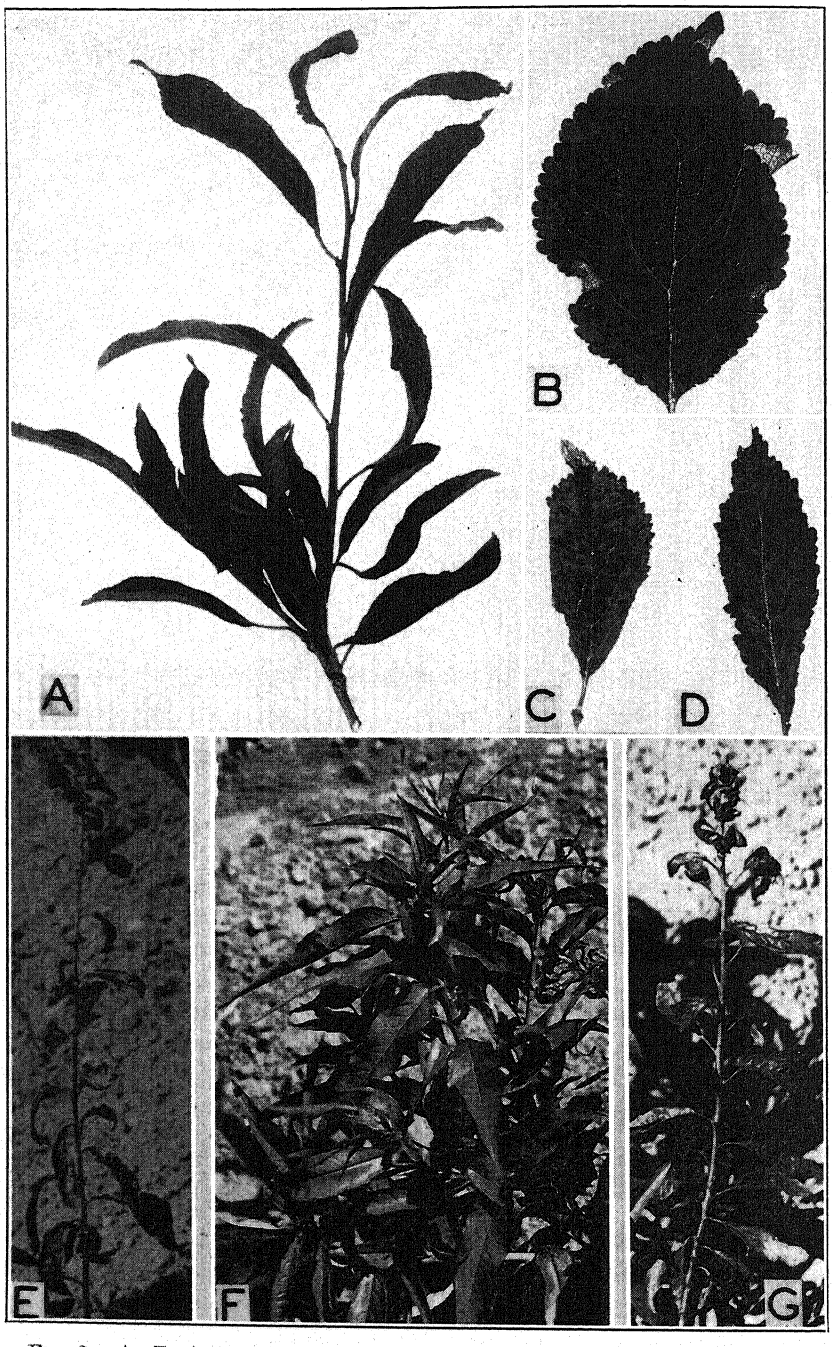


FIG. 2. A: Typical symptoms of strain 1 on Italian prune; B, C: strain 2 on Lombard showing wavy margins (B) and marking and narrowing of leaves (C); D: strain 4 in Lombard; E: strain 3 on Italian prune; F: strain 1 on peach seedling; G: strain 4 on Italian prune.

On Grand Duke plum.—Only strains 1 and 2 were used for inoculating Grand Duke in 1942. This variety was little affected by 1, traces of mottle and ring spotting appearing on the first few leaves. Strain 2, though having little or no effect on vigor, produced quite striking line patterns and some oak-leaf which persisted throughout the summer (Fig. 3, L).

On Abundance plum.—Although a few, faint, transient, pale green rings appeared on the first few leaves of one Abundance shoot inoculated with strain 1 and faint chlorotic areas on other plants inoculated with strains 1, 3, and 4, these symptoms were too illusive to be diagnostic. Evidence of transmission of strain 2 was only slightly more satisfactory, as traces of oak-leaf and other patterns were observed on the younger leaves in July. In 1943, therefore, healthy Italian prune buds were grafted in the Abundance to test for the presence of prune dwarf. Results of this budding of course will not be available until the growing season of 1944.

This clon of Abundance, though itself quite healthy in appearance, when indexed on peach, gave some indications that it carried a virus of the peach-yellows-little-peach group.

On peaches.—Though the differences between the strains of prune dwarf tended to be more apparent on peaches than in some other hosts, there has been little variation in the reaction of the different peach varieties to any one strain of the virus. Minor differences in the degree of symptom expression have been encountered among seedlings, but, for present purposes, a description of the effects of each prune dwarf strain can be applied to peaches generally.

Strain 1 characteristically, especially in the first season, produced a slight delay in foliation and a moderate dwarfing by reducing both number and length of internodes (Fig. 2, F). Some early leaves showed faint marking, such as mottles, chlorotic spots, feathery patterns, and confluent rings, which disappeared later in the season. The newer leaves were often loosely rolled upward and inward from the margins and were more or less stiff and upward-pointing or had wavy margins (Fig. 2, F). Superficial bark necrosis sometimes occurred near the point of inoculation. Trees affected with strain 1 tended to recover after the first season.

The symptoms with strain 2 were of the same nature but were always more severe, while the leaf markings, usually a fine network of small rings and fine lines, persisted throughout the summer. The original inoculation to Elberta resulted in a very pronounced shortening of internodes amounting almost to a loose rosette. The leaves were narrower, smaller, and stiffer than normal, the bark became roughened, and the condition of the tree deteriorated from year to year. In sub-inoculations from this tree, reactions were somewhat milder and there was slight recovery in the second and third seasons, suggesting that the strain had lost some of its aggressiveness after passage through peach.

Strain 3, on the other hand, was generally less dwarfing than 1 though resembling that strain in other respects. On some seedlings, the symptoms of 3 were almost suppressed.

The original inoculations with strain 4 on seedlings induced a dwarfing almost as severe as that caused by strain 2, but with sub-inoculation strain 4 became intermediate between strains 1 and 2 in its dwarfing effect. Leaf markings like those described for strain 1 appeared only on early leaves, but strain 4 frequently imparted a yellow cast to the foliage by exaggerating the "growth mottle" sometimes seen on the leaves of healthy peach trees. Normally the "growth mottle" consists of pallid interveinal tissues outlined by dark green veins and is usually outgrown as the leaf matures. On the trees affected by strain 4, the pallor was heightened to a fairly vivid and persistent yellow.

On Montmorency cherry.—As all cherries in the double-budding experiments of 1940 and 1941 failed to grow, only one season's observations, on the 1942 series, have been available for any of the cherry varieties. The only symptoms of strain 1 on Montmorency were some pale green rings and mottle and traces of fine brownish lines on the early formed leaves. Later leaves were symptomless.

With strain 2, there was a considerable amount of pronounced mottle, ringing, and chlorotic or necrotic spotting on some early leaves (Fig. 3, D) and on others a network of fine brown rings, lines (Fig. 3, E), and flecks (Fig. 3, F) which sometimes became necrotic. In July, line patterns were seen on some leaves but they had disappeared a month later. Strain 2 also appeared to have a slight dwarfing effect.

Strain 3 caused only a faint, coarse mottle early in the season. Even these symptoms were rather indefinite and transient.

Strain 4 gave rise to slight delay in foliation and to considerable coarse mottle and ringing and to some necrotic spotting and flecking on early leaves only. In its reaction on Montmorency, 4 was thus intermediate between strains 1 and 2.

On Napoleon cherry.—All four strains induced various combinations and intensities of large ring and oak-leaf patterns on some leaves in early spring (Fig. 3, C). They were faint with strain 3, somewhat plainer with 1, and fairly well marked with 2 and 4, and more or less transient with all. In the case of strains 2 and 4, oak-leaf patterns appeared on a few new leaves as late as the beginning of July. All strains except strain 1 may have caused a slight stunting but it was impossible to be sure of this point. The buds in the vicinity of strain 3 inoculations were somewhat slow in opening.

On Black Tartarian cherry.—Symptoms on Black Tartarian were, if anything, less conspicuous than on Napoleon. Strain 3 was almost completely masked, while the markings of strain 1, if present at all, were faint on early leaves. With strain 2 the coarse-ring, line, and oak-leaf patterns were few and mild though apparent on new leaves until July (Fig. 3, A). Strain 4 symptoms were much the same as, though somewhat plainer than, those of 2 and persisted on the older leaves for some time (Fig. 3, B).

On Black Eagle cherry.—Black Eagle, inoculated with strains 1, 3, or 4 exhibited a faint mottle early in the spring, and with strain 2 some rings and coarse lines, but when indexed on peach and Mahaleb seedlings was itself

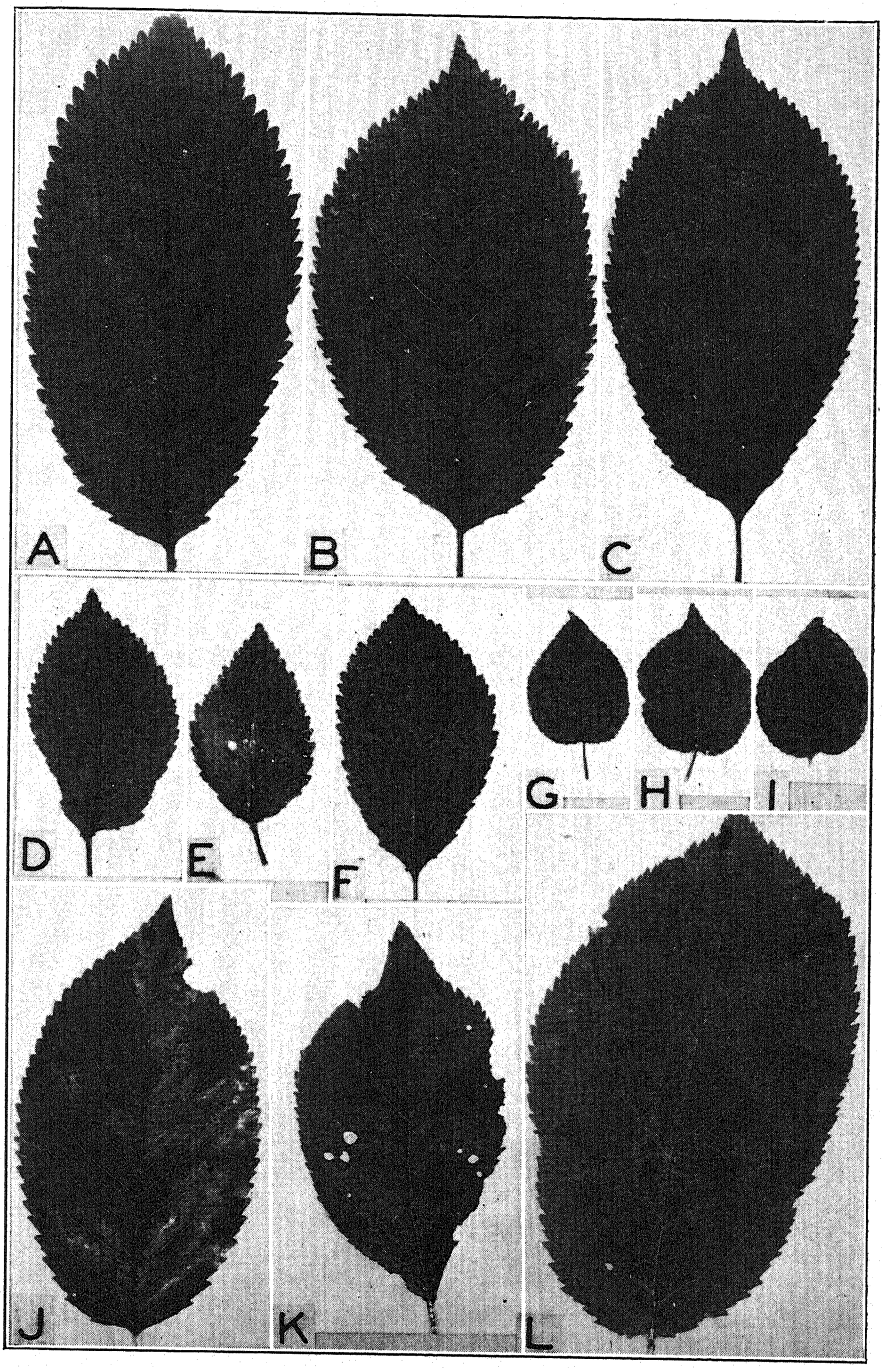


FIG. 3. A: strain 2 and (B) strain 4 on Black Tartarian; C: strain 4 on Napoleon; D–F: strain 2 in Montmorency, D: mottle and rings, E: brown lines and rings, F: brown flecking; G–I: typical symptoms on Mahaleb, G: lines and small rings, H: coarse rings, I: vein-banding; J: symptoms on Elkhorn source of strain 3; K: strain 2 on German prune; L: strain 2 on Grand Duke.

shown to be already virus-infected. The leaf markings on Eagle shoots, however, were in all probability induced by the prune dwarf virus as they were of the same order as those on the other sweet cherry varieties and appeared only on inoculated shoots.

The peach seedlings used for indexing this Black Eagle clon showed both fine and coarse rings and oak-leaf patterns, but the most striking symptom appeared in June as a necrotic streak down one side of each main shoot, near the tip, which was bent over to form a crook. Later the whole tip died. Indexed Mahaleb seedlings were somewhat dwarfed and their leaves were marked with faint oak-leaf and coarse ring patterns in mid-season. The identity of this virus is yet to be established, but it has none of the usual earmarks of prune dwarf.

On Elkhorn cherry.—This variety had either no symptoms or a faint mottle on early leaves. Unfortunately, indexing demonstrated that this supposedly healthy Elkhorn was already carrying a virus presumed to be prune dwarf virus on the basis of the symptoms on indexed peach. If this diagnosis is correct, the virtual absence of symptoms in the inoculated Elkhorn series is understandable since the tree, from which the buds were taken, had been selected as healthy and therefore either had always been symptomless or had long recovered from the initial shock.

On Niagara apricot.—There were no characteristic leaf symptoms of diagnostic value on apricot although, on some of the shoots inoculated with strain 2 or strain 3, some leaves had faint mottling or chlorotic spotting or streaking in the early part of the season. All strains and more especially 1 and 2 induced delay in foliation. Reduction in vigor and growth was considerable with strains 2 and 4 and somewhat less with 1 and 3. As a matter of interest and possibly of significance, two of the three apricot shoots inoculated with strain 2 died during the summer. The fact that, barring two buds that failed to take in other series, these were the only cases of mortality in the apricot experiment suggests that the more virulent strain 2 was responsible. A similar lethal effect with strain 2 has already been mentioned in connection with the double-budding experiment with Italian prune.

On Myrobalan plum.—Myrobalan seedlings exhibited no definite symptoms except with strain 2 when chlorotic mottles, lines, and rings appeared occasionally on early leaves and some plants were more or less stunted. Prune dwarf virus, however, can be transmitted through Myrobalan.

On Mahaleb cherry.—Mahaleb seedlings were quite variable in their reaction. Some were almost symptomless and some had early leaves marked with mottles, lines (Fig. 3, G), coarse rings (Fig. 3, H), and occasionally vein-banding (Fig. 3, I). With strain 2 and to a lesser extent with strains 1 and 3, coarse rings and oak-leaf patterns also appeared on leaves developed late in the summer.

DISCUSSION

In view of the striking similarity of the symptoms of these four viroses on each of the hosts which manifest them, and particularly on Italian prune,

Lombard plum, and peach, little argument is necessary to justify the thesis already proposed that all four are caused by strains of one virus. As a matter of fact, if any distinctions are to be made, there are greater differences between the two strains from plum than between the plum and the cherry strains. Prune dwarf, whether the causal strains of virus be considered individually or collectively, is manifested by symptoms of one or more of the following categories: (a) stunting or reduction of vigor; (b) leaf distortion; (c) patterns on leaves; and (d) crop reduction. The present experiments are not yet of sufficient duration to provide many yield data, but Hildebrand (8, 14) has presented some evidence on that point. With regard to the other types of symptoms, however, the combination exhibited has been much more specific to the host than to the strain.

It should also be observed that, on virtually every host in the range of these experiments, the strains if arranged according to increasing severity of effect would fall in the same order, namely: 3, 1, 4, and 2. Consequently differences occurring between strains, for example, the pattern-forming capacity so pronounced in 2, may be ascribed to differences in virulence. The markings on some hosts, especially with strain 2, bear some resemblance to those induced by the virus of line pattern (1, 3), but some transmission experiments with Shiro plum, which is one of the best indicators of line pattern (1, 9) and with line pattern on the host range, failed to show that that virosis is related to prune dwarf though its virus may be involved as a contaminant in strain 2.

Each species of *Prunus* and, in some instances, each variety has its characteristic reaction to prune dwarf. The typical response of peaches is some degree of dwarfing usually by internode shortening, mild leaf distortion in the form of wavy margins, and foliage patterns of varying intensities and persistence. In cherries, leaf patterns are almost exclusively predominant, at least as primary symptoms, and there is good evidence that some varieties of sweet cherries can become symptomless, or nearly so, with the possible exception of crop reduction. For that matter, *P. mahaleb* and, to a lesser degree, both sweet and sour cherries are not satisfactory differential hosts for prune dwarf because they respond to a number of distinct viroses with confusingly similar markings. Plums have so wide a range of reaction that they can be divided into at least three groups: (a) severely affected varieties, such as Italian prune and Lombard, in which leaf distortion and stunting of growth are the most striking symptoms; (b) moderately or slightly affected varieties, such as Reine Claude, German prune, and Grand Duke, which show almost no leaf distortion and little stunting but some leaf marking; and (c) varieties, such as Abundance and Damson, in which symptoms rarely occur.

The fact that some symptoms, especially leaf patterns, tend to occur only early in the season suggests that the degree of symptom expression may be affected by temperature or other conditions during the period of leaf formation. On the other hand, recovery in the second season would indicate

that, with some hosts at least, infection has an initial shock effect which is not sustained. Both factors are probably operative, though it is not yet possible to assess their relative significance.

The findings recorded here with respect to the effect of prune dwarf on German prune do not agree with those of Hildebrand (8). This discrepancy may have arisen because of differences in the strains of virus employed, but both Hildebrand's failure to obtain symptoms on cherries (8) and his description of peach symptoms would suggest that he was working with a comparatively mild strain and the mild strains in Ontario had little effect on German prune. So it would appear either that different strains of that variety were used in the two investigations or that there was some confusion regarding the identity of German prune in New York State and in Ontario. If either is true and because the same sort of situation might easily arise with other host varieties, stone fruit virus investigations would benefit from some form of standardization of experimental varietal material.

The similarity of the symptoms of prune dwarf on peach to those of rosette mosaic (2, 4, 7) has prompted Hildebrand (7, 8) to suggest a possible relationship between the two viruses. While conclusive proof is yet to be established, it is interesting to note another point of resemblance in that Cation (4) reported transmission of a disease which he diagnosed as rosette mosaic from Damson to peach. The demonstration of strain differences in both diseases opens up the question still further. Whether or not the virus of rosette mosaic is a strain of prune dwarf virus, the natural occurrence of masked forms of prune dwarf on Damson and its presence in sweet cherry twice and probably three times observed serve to indicate that this disease is more wide spread than has yet been realized.

The author wishes to express his sincere thanks to Dr. G. H. Berkeley for his unfailing interest and many helpful suggestions in connection with this investigation.

SUMMARY

Four viroses have been studied in Ontario, two on plum and two on sweet cherry. Symptomatological evidence is presented that the viruses are strains of prune dwarf virus (Prunus virus 6; *Nanus pruni* H.), differing in virulence. All four strains have been transmitted to at least four varieties of *Prunus domestica,* one of *P. salicina,* two of *P. persica,* one of *P. cerasus,* two of *P. avium,* one of *P. armeniaca, P. cerasifera* (Myrobalan), and *P. mahaleb.*

All strains produced typical strap-shaped, rugose, thickened leaves and dwarf growth on Italian prune and Lombard plum; stunting and leaf patterns on peach varieties; and ring, line, and oak-leaf patterns on cherries and *P. mahaleb.* Some plum varieties, namely Reine Claude, German prune, and Grand Duke, showed leaf markings without distortion while some were almost symptomless. Myrobalan was symptomless except with the most virulent strain which induced chlorotic mottles, lines and rings, and sometimes dwarfing.

A tendency to recovery was noted in peaches and cherry varieties after the initial shock symptoms had appeared.

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PHYSIOLOGIC SPECIALIZATION AND THE CONTROL OF MILLET SMUT[1]

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INTRODUCTION

Smut, caused by *Ustilago crameri* Kcke., is one of the important diseases of millet, *Setaria italica* (L.) Beauv., and is coextensive with the cultivation of the crop. In China, millet is one of the principal food crops, the annual production being approximately 28,675,548,000 pounds (1), and the losses caused by the smut are often of considerable economic importance. Reductions in yield of from 10 to 50 per cent in Northern and Northeastern China are not uncommon (6).

Although millet smut can be controlled effectively by proper seed treatment, obviously it would be more desirable and economical to control it by means of resistant varieties. Most of the common varieties of millet so far tested are susceptible; therefore, selection of lines or the breeding of varieties with suitable agronomic characters and resistance to smut would be necessary. As an aid to the breeding program, it is important to learn the number and geographic distribution of physiologic races of the smut fungus and their pathogenicity on the most important varieties. While the ultimate control of millet smut by breeding resistant varieties is being sought, any control which can give immediate relief, such as seed treatment, also is worthy of attention. Therefore, the relative efficacy of new and old seed disinfectants for the control of millet smut was investigated.

PHYSIOLOGIC SPECIALIZATION IN USTILAGO CRAMERI

Physiologic specialization in certain smut fungi has been known for a long time (2, 9), although it has not been recorded for *Ustilago crameri*. It seemed advisable to determine the reactions of the common varieties of millet to various collections of the millet smut.

Varieties of millet were obtained from the Department of Agronomy, College of Agriculture, Honan University, Honan, China, and from the Division of Agronomy and Plant Genetics, University Farm, St. Paul, Minnesota. All the varieties except German No. 18, German Dark, and Siberian No. 13020, were selfed for several years prior to these tests.

Seed lots were soaked for 8 minutes in 1:250 solution of formaldehyde, covered for 4 hours, washed in water for 4 hours, and then allowed to dry thoroughly. The seed was inoculated by applying an equal amount of dry chlamydospores to each lot of seed, about 0.5 g. of smut spores to 100 g. of seed.

[1] A portion of a thesis presented to the Graduate School, University of Minnesota, June, 1937, in partial fulfillment of the requirements for degree of Doctor of Philosophy. Published as paper 2169 of the Journal Series of the Minnesota Agricultural Experiment Station.

During the winter of 1935, preliminary pathogenicity tests were made in the greenhouse with 5 collections of *Ustilago crameri* on 4 varieties of millet. Although the number of plants inoculated was small, the results indicated clearly that the 5 collections of smut differed in their virulence on 4 varieties of millet. On the basis of these preliminary results, more extensive varietal tests were made with more collections of smut.

In the summer of 1935, 11 varieties of millet were inoculated with 9 collections of *Ustilago crameri*, which were obtained from different parts of China. All the varieties of millet tested were planted at University Farm,

TABLE 1.—*Percentage of smutted heads in 12 varieties of millet inoculated with 9 collections of Ustilago crameri, grown in St. Paul, Minnesota, in 1935 and 1936*

Variety of millet	Year	Percentage of smutted heads[a] with different smut collections									
		5	6	8	9	10	12	13	14	17	Con-trol
German No. 10	1935	0	0	0	0	0	0	19	47	3	0
	1936	0	0	0	0	2	0	4	37	10	0
German No. 18	1935	60	51	52	79	73	71	85	63	55	0
	1936	68	35	47	81	42	65	66	39	70	0
German Dark	1935	33	62	42	90	83	88	24	64	63	0
	1936	47	73	38	82	91	77	40	66	76	0
Hungarian No. 232	1935	50	75	59	86	89	81	52	93	81	0
	1936	57	67	53	77	70	64	68	82	57	0
Kaifeng No. 142	1935	63	73	57	61	83	98	51	100	92	0
	1936	67	84	66	69	68	91	70	87	58	0
Kaifeng No. 232	1935	26	51	33	58	65	0	39	0	0	0
	1936	39	69	21	56	77	2	32	0	0	0
Nanking No. 31	1935	5	7	0	0	1	0	16	14	1	0
	1936	0	13	0	0	16	8	43	6	6	0
Teipien No. 48	1935	44	65	58	56	71	75	40	79	39	0
	1936	57	58	16	35	60	57	44	61	48	0
Unnamed Selection No. 11	1935	83	79	75	86	90	89	57	93	96	0
	1936	68	78	58	53	86	76	59	44	83	0
Unnamed Selection No. 12	1935	48	63	34	59	77	87	8	54	36	0
	1936	74	87	43	64	62	72	26	50	30	0
Siberian No. 1120	1935	68	90	77	82	87	84	60	76	53	0
	1936	63	73	66	70	69	49	50	61	55	1
Siberian No. 13020	1936	6	10	40	2	17	58	31	27	26	0
Race No.		1	1	2	1	1	3	4	5	6	

[a] Percentage computed on number of heads in duplicate rows. (The number of heads in each row ranged from 300 to 400.)

St. Paul, Minnesota, in duplicate 5-foot rows, one foot apart, in a split-plot arrangement. The smut percentages were based on counts of 300 to 400 heads in a row. In 1936 similar tests were made, including an additional variety. The results for the two years' tests are summarized in table 1.

There are striking differences in pathogenicity among the collections of smut. For instance, collection 14 produced no smut on the variety Kaifeng No. 232, but more than 85 per cent infection on the variety Kaifeng No. 142; whereas collection 10 of *Ustilago crameri* caused between 65 and 83 per cent infection on these two varieties.

In general, the pathogenicity of a collection on a particular variety proved to be similar in 1935 and in 1936. Fluctuation in the percentages of smut from one season to another is to be expected, because environmental factors may influence profoundly the development of smuts. It is possible that certain collections of smut consisted of many biotypes that differed greatly in parasitic capabilities and in response to environmental conditions.

In a given year all varieties of millet were grown under parallel conditions; hence differences in their reaction to a smut collection must be attributed to differences in virulence of the latter. This certainly would be true of the test made in 1936, as the inoculum and seed sown were produced and stored under identical conditions and the seed was sown the same day in the experimental plots at St. Paul, Minnesota.

Since each collection of smut in 1936 differed somewhat in parasitism, each one might be considered a physiologic race. This fine distinction seems undesirable and also impractical, however, because of the wide fluctuation in percentages of smut that occurred on the same varieties in different years. Therefore, the determination of races was based on fairly wide differences in pathogenicity.

There are at least 6 distinct parasitic races of *Ustilago crameri* (Table 1). Collections 5, 6, 9, and 10 are classified as race 1, because of their similarity in virulence on the 5 differential hosts: German No. 10, Kaifeng No. 232, Nanking No. 31, Unnamed Selection No. 12, and Siberian No. 13020. Each of the other 5 collections consisted of a different physiologic race. Although the separation of collections into races was more or less arbitrary, consideration was given to variation in smut percentages produced by the same races on a given variety in different replications and in different years. The important point is that there are many races of *Ustilago crameri* that must be considered in breeding smut resistant varieties of millet.

Of course, it is possible that, if further tests were made, certain collections that now appear distinct would be grouped together; also it is possible that certain collections might consist of two or more races. Therefore, the separation of collections into races must be considered of a temporary nature.

All varieties of millet tested were susceptible to certain races of *Ustilago crameri* (Table 1). Although a few varieties were highly resistant to several of the races, most of them were susceptible to 3 or more races. German No. 10 was the most resistant variety tested, while Hungarian No. 232, Siberian No. 1120, and German No. 18 were among the most susceptible varieties and were attacked by all the races.

EFFECTIVENESS OF VARIOUS FUNGICIDES IN CONTROLLING MILLET SMUT

The earliest experiments on the control of millet smut by various fungicides were made by Hecke (3, 4). He used copper sulphate, sulphuric acid, mercury bichloride, and formalin; and, in addition, a hot water treatment. Subsequently, many fungicides were tested by several workers (5, 7, 8). The most promising ones were copper carbonate, formaldehyde dip, and

copper sulphate. New Improved Ceresan, a good fungicide for many cereal smuts, apparently has not been tried.

In studying the effectiveness of various fungicides in controlling millet smut, a moderately susceptible variety, Kaifeng No. 232, which had been selfed for 3 years, was used. The inoculum was a mixture of chlamydospores of the 10 collections of *Ustilago crameri*. Prior to treatment, the millet seeds were cleaned and then artificially inoculated with a mixture of chlamydospores of *Ustilago crameri*. The various fungicides and the rate of application are given in table 2. For each treatment, except formaldehyde solution, the amount of fungicide required was first put in a flask and shaken thoroughly; then the seed was added and the flask was shaken again, until the dust covered the seeds thoroughly. When formaldehyde solution

TABLE 2.—*Results of treating Kaifeng millet with 10 fungicides when seed was inoculated artificially with chlamydospores of 10 collections of Ustilago crameri, in 1935 and 1936*

Fungicide		Year and percentage of infection[b]	
Name	Ounces per bushel	1935	1936
New Improved Ceresan	0.5	0.8	0.4
Formaldehyde dip[a]	.....	1.0	1.1
Cuprocide	2.0	3.6	1.0
Copper carbonate (20 pct.)	4.0	3.1	3.1
Barbak III	2.0	.....	4.6
Kopper's flotation sulphur dust	2.0	5.0	10.9
Powdered sulphur	4.0	.....	15.8
Gas sulphur	2.0	16.6	14.2
Sulphur dust	4.0	23.6	18.8
Formaldehyde dust	3.0	29.7	39.7
Powdered sulphur	2.5	36.2	32.2
Check	0.0	26.9	42.9

[a] The concentration used was 1 part formaldehyde to 250 parts of water.
[b] Results were based on 400–500 heads grown in quadruplicate plots. The minimum level for statistical significance between any two means was 0.74 in 1935 and 1.45 in 1936.

was used, the seed was soaked in the solution for 7 minutes, covered for 5 hours, and then washed and dried in the usual manner.

Nine fungicides were tested in 1935, eleven in 1936. In both seasons, the tests were in triplicate, 10-foot-row plots, replicated 4 times and randomized. The percentages of smut were based on the number of heads infected. The results are in table 2.

In 1935 all fungicides used, except powdered sulphur used at the rate 2.5 oz. per bushel and formaldehyde dust, tended to reduce the percentage of infection, but none of them eliminated smut completely. New Improved Ceresan and formaldehyde dip were more effective than the others, although several other fungicides also were partially effective.

The results in 1936 were similar to those in 1935, except for slight differences. There was no apparent stimulation or increase of the percentage of infection when powdered sulphur and formaldehyde dust were used.

In order to determine the statistical significance of the effectiveness of the different fungicides tested, the data for 1935 and 1936 (Table 2) were analyzed separately by the analysis of variance method. The minimum level for statistical significance between any two means was 0.74 in 1935 and 1.45 in 1936. New Improved Ceresan and formaldehyde dip were the most effective fungicides in 1935; cuprocide and copper carbonate were next. Kopper's flotation sulphur dust was more effective than any other sulphur fungicides.

DISCUSSION

Millet is a food crop which is exceeded in importance in Northern China only by wheat. Smut caused by *Ustilago crameri* is one of the most important diseases of millet. Because of the importance of the crop and the losses caused by this smut, its control is of major importance. While it can be fairly well controlled by certain seed treatments, the development of resistant varieties will be very important in China, where it often is difficult to induce farmers to treat the seed.

Because of the importance of physiologic races of smuts in breeding disease resistant varieties of cereals, the writer investigated the question of physiologic specialization in *Ustilago crameri*. In 1935 eleven varieties of millet were inoculated with 9 collections of smut from China. These collections differed greatly in their pathogenicity. In 1936 the tests were repeated, using one additional variety of millet, and similar differences occurred.

The differences in the reactions to the 9 collections in 1935 and 1936 are not unexpected. Chlamydospore collections may contain many biotypes, and, furthermore, recombinations can be expected. Also, it is well known that differences between biotypes or groups of biotypes may become apparent under some conditions and not under others. In spite of the fluctuation in percentages of smut in certain cases, there are at least 6 distinct parasitic races of *Ustilago crameri*. This fact is of great importance in breeding disease resistant varieties. Although there were great differences in reaction of millet varieties to different races, none of those tried was resistant to all the smut races. Consequently further search must be made for resistant varieties.

SUMMARY

Nine collections of *Ustilago crameri* were tested for their virulence on 12 varieties of millet. Of these, 6 differed sufficiently in pathogenicity on 5 differential hosts to be designated as physiologic races.

Although certain varieties of millet were highly resistant to several races, no variety was resistant to all races.

The prevalence of races of smut must be considered in breeding millet varieties resistant to the disease.

Eleven fungicides were tested as seed disinfectants, and all except powdered sulphur and formaldehyde dust reduced smut. New Improved

Ceresan was very effective; formaldehyde dip, cuprocide, copper carbonate, and Barbak III were fairly effective. In general, sulphur fungicides were not effective.

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PARASITISM OF RHIZOCTONIA SOLANI ON BEANS[1]

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In most parts of the United States, and especially in the South where moisture and temperature conditions are favorable, many species of plants are attacked by fungi belonging to the genus *Rhizoctonia*. While these fungi are a heterogeneous group, many of them, because of certain sclerotial characteristics, are considered by most investigators to be *Rhizoctonia solani* Kühn.

That *R. solani* comprises distinct strains differing in morphology, cultural characters, and pathogenicity has been shown by many investigators. Among them are Peltier (12), Rosenbaum and Shapovalov (14), Edson and Shapovalov (2), Matsumoto (9), van der Meer (10), Walker (18), Lauritzen (7), Wiant (19), Gratz (6), Elmer (3), LeClerg (8), Briton-Jones (1), Monteith and Dahl (11), and Sanford (15). The more recent studies of LeClerg (8) and Sanford (17), in which large numbers of isolates from Irish potatoes were tested for pathogenicity on sugar beets, indicated that nearly all were nonpathogenic on this host. These results are of particular interest when certain control problems, such as crop rotation, are being considered.

In Louisiana the common garden bush snapbean, *Phaseolus vulgaris* L., is often attacked by *Rhizoctonia*. Stands are reduced and reddish-brown lesions develop on the stems near the soil surface. As these fungi occur on many hosts other than beans in this State, it seemed desirable to determine if isolates from other hosts were pathogenic on beans.

To obtain this information, a number of isolates were secured from various hosts in Louisiana and from other investigators. Some studies were made of the cultural characters and reactions to temperature of the various cultures, but the major part of the investigations was devoted to a study of their pathogenicity on beans.

ORIGIN OF ISOLATES

From a rather large number of isolates, 27 were finally selected for these studies. Pertinent information regarding them is in table 1.

CULTURAL CHARACTERS

The isolates were compared on bean and potato-dextrose agar. Differences occurred in such characters as the color of the mycelium, rate of growth, and sclerotium formation. A representative group of 6 isolates is shown in figure 1.

[1] Summary of part of a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted by the University of Minnesota, August, 1937.

EFFECT OF TEMPERATURE ON RATE OF GROWTH

Twelve isolates from 8 hosts differed considerably in rate of growth on potato-dextrose agar in Petri dishes at 7 different temperatures (Table 2). Only a trace of growth occurred at 6° C. in the isolates from tomato (R7), eggplant (R6), and bean (R76), as compared to 9 to 17 mm. with the other 9 cultures. The optimum temperatures also differed, being from 20° to

TABLE 1.—*Origin of isolates of Rhizoctonia solani*

Number of isolate	Host plant	Part of plant from which isolated	Location	By whom isolated	Date of isolation
R3	Sugar cane	Root	Baton Rouge, La.	E. C. Tims	1932
R4	do	do	do	do	1932
R5	do	do	do	do	1932
R6	Eggplant	Stem base	do	S. Smith	1933
R7	Tomato	do	do	do	1933
R8	Potato	do	do	do	1933
R9	Bean	do	Houma, La.	L. H. Person	1931
R62	Sugar beet	—	Chaska, Minn.	E. L. LeClerg	1930
R64	Bean	Stem base	Houma, La.	L. H. Person	1931
R76	do	do	Baton Rouge, La.	do	1931
R77	do	do	Houma, La.	do	1932
R100	Sugar beet	Seedling	Ohio	E. L. LeClerg	1930
R110	Potato	Tuber (sclerotium)	St. Paul, Minn.	do	1930
R115	do	Stem	Guthrie, Minn.	do	1931
R120	Pea	Root	St. Paul, Minn.	G. H. Starr	—
R127	Potato	Stem	Dilworth, Minn.	E. L. LeClerg	1931
R128	do	do	do	do	1931
R150	Potato	Tuber (sclerotium)	Amherst, Mass.	do	1933
R162	do	do do	Scotts Bluff, Neb.	do	1933
R170	do	do do	Hay Springs, Neb.	do	1933
R178	do	— —	California	do	1933
R188	Rice	Seedling	Welch, La.	D. E. Ellis	1932
R191	Potato	— —	Topeka, Kansas	E. L. LeClerg	1933
R194	Rice	Seedling	Welch, La.	D. E. Ellis	1932
R200	Potato	Tuber (sclerotium)	Kentville, Nova Scotia	E. L. LeClerg	1933
R208	Sugar cane	Stalk (interior)	Baton Rouge, La.	E. C. Tims	1934
R310	Pea	— —	Moscow, Idaho	W. H. Pierce	1935

25° C. for the isolate from potato (R8) and from 25° to 30° C. for the others. Isolates from sugar cane (R4), bean (R9), and sugar beet (R100) grew slowly at 38° C., while the others did not grow.

METHODS USED IN PATHOGENICITY TESTS

For the pathogenicity tests, isolates were grown on a sterile mixture of 4 parts oats and 1 part wheat to which water was added at the rate of 80 per cent by weight. The cultures used for inoculum usually were from 4 to 6 days old when the inoculations were made.

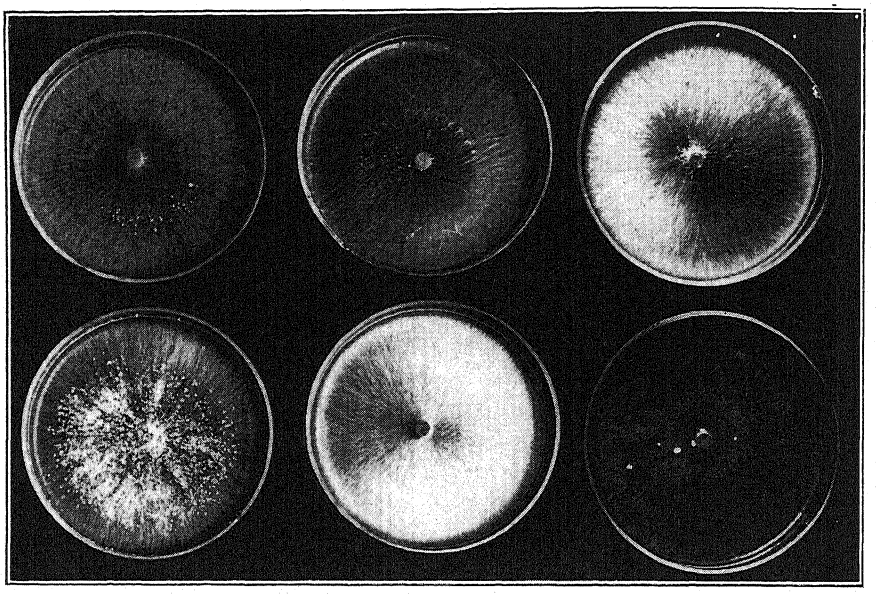


FIG. 1. Six culturally different isolates of *Rhizoctonia solani*.

Inoculations were made in the greenhouse in sterilized soil and in the field. The greenhouse inoculations were of two types: soil inoculation and direct seedling inoculation. The soil was inoculated by mixing approximately 25 grams of the inoculum into 6-inch pots of sterilized soil. After 4 to 6 days the soil was remixed and the seeds were planted one inch deep. Bean[2] seedlings were inoculated directly by removing the soil from the base of the stem and placing the inoculum against the basal-stem portion. The soil was then replaced, covering the inoculum.

Seedlings were inoculated directly in the field similarly to those in the

TABLE 2.—*Effect of temperature on the rate of growth of 12 isolates of Rhizoctonia solani on bean agar (average of 3 tests of 3 replications each)*

Original host	Isolate number	Average diameter of colonies in mm. after 48 hours						
		6° C.	15° C.	20° C.	25° C.	30° C.	34° C.	38° C.
Sugar cane	R4	10	22	37	61	62	54	8
Potato	R8	14	26	36	37	22	11	0
Rice	R194	14	26	43	80	78	69	0
Pea	R120	17	35	51	71	54	23	0
Pea	R310	16	31	49	73	52	26	0
Bean	R9	10	30	47	80	78	57	8
Bean	R64	9	30	51	86	85	43	0
Bean	R76	1	32	50	85	79	48	0
Tomato	R7	1	26	40	69	75	64	0
Eggplant	R6	1	30	47	87	83	66	0
Sugar beet	R62	12	21	35	52	43	23	0
Sugar beet	R100	11	22	39	65	59	43	10

[2] Unless otherwise stated, the term bean refers to bunch snapbeans (*Phaseolus vulgaris*).

On Grand Duke plum.—Only strains 1 and 2 were used for inoculating Grand Duke in 1942. This variety was little affected by 1, traces of mottle and ring spotting appearing on the first few leaves. Strain 2, though having little or no effect on vigor, produced quite striking line patterns and some oak-leaf which persisted throughout the summer (Fig. 3, L).

On Abundance plum.—Although a few, faint, transient, pale green rings appeared on the first few leaves of one Abundance shoot inoculated with strain 1 and faint chlorotic areas on other plants inoculated with strains 1, 3, and 4, these symptoms were too illusive to be diagnostic. Evidence of transmission of strain 2 was only slightly more satisfactory, as traces of oak-leaf and other patterns were observed on the younger leaves in July. In 1943, therefore, healthy Italian prune buds were grafted in the Abundance to test for the presence of prune dwarf. Results of this budding of course will not be available until the growing season of 1944.

This clon of Abundance, though itself quite healthy in appearance, when indexed on peach, gave some indications that it carried a virus of the peach-yellows-little-peach group.

On peaches.—Though the differences between the strains of prune dwarf tended to be more apparent on peaches than in some other hosts, there has been little variation in the reaction of the different peach varieties to any one strain of the virus. Minor differences in the degree of symptom expression have been encountered among seedlings, but, for present purposes, a description of the effects of each prune dwarf strain can be applied to peaches generally.

Strain 1 characteristically, especially in the first season, produced a slight delay in foliation and a moderate dwarfing by reducing both number and length of internodes (Fig. 2, F). Some early leaves showed faint marking, such as mottles, chlorotic spots, feathery patterns, and confluent rings, which disappeared later in the season. The newer leaves were often loosely rolled upward and inward from the margins and were more or less stiff and upward-pointing or had wavy margins (Fig. 2, F). Superficial bark necrosis sometimes occurred near the point of inoculation. Trees affected with strain 1 tended to recover after the first season.

The symptoms with strain 2 were of the same nature but were always more severe, while the leaf markings, usually a fine network of small rings and fine lines, persisted throughout the summer. The original inoculation to Elberta resulted in a very pronounced shortening of internodes amounting almost to a loose rosette. The leaves were narrower, smaller, and stiffer than normal, the bark became roughened, and the condition of the tree deteriorated from year to year. In sub-inoculations from this tree, reactions were somewhat milder and there was slight recovery in the second and third seasons, suggesting that the strain had lost some of its aggressiveness after passage through peach.

Strain 3, on the other hand, was generally less dwarfing than 1 though resembling that strain in other respects. On some seedlings, the symptoms of 3 were almost suppressed.

The original inoculations with strain 4 on seedlings induced a dwarfing almost as severe as that caused by strain 2, but with sub-inoculation strain 4 became intermediate between strains 1 and 2 in its dwarfing effect. Leaf markings like those described for strain 1 appeared only on early leaves, but strain 4 frequently imparted a yellow cast to the foliage by exaggerating the "growth mottle" sometimes seen on the leaves of healthy peach trees. Normally the "growth mottle" consists of pallid interveinal tissues outlined by dark green veins and is usually outgrown as the leaf matures. On the trees affected by strain 4, the pallor was heightened to a fairly vivid and persistent yellow.

On Montmorency cherry.—As all cherries in the double-budding experiments of 1940 and 1941 failed to grow, only one season's observations, on the 1942 series, have been available for any of the cherry varieties. The only symptoms of strain 1 on Montmorency were some pale green rings and mottle and traces of fine brownish lines on the early formed leaves. Later leaves were symptomless.

With strain 2, there was a considerable amount of pronounced mottle, ringing, and chlorotic or necrotic spotting on some early leaves (Fig. 3, D) and on others a network of fine brown rings, lines (Fig. 3, E), and flecks (Fig. 3, F) which sometimes became necrotic. In July, line patterns were seen on some leaves but they had disappeared a month later. Strain 2 also appeared to have a slight dwarfing effect.

Strain 3 caused only a faint, coarse mottle early in the season. Even these symptoms were rather indefinite and transient.

Strain 4 gave rise to slight delay in foliation and to considerable coarse mottle and ringing and to some necrotic spotting and flecking on early leaves only. In its reaction on Montmorency, 4 was thus intermediate between strains 1 and 2.

On Napoleon cherry.—All four strains induced various combinations and intensities of large ring and oak-leaf patterns on some leaves in early spring (Fig. 3, C). They were faint with strain 3, somewhat plainer with 1, and fairly well marked with 2 and 4, and more or less transient with all. In the case of strains 2 and 4, oak-leaf patterns appeared on a few new leaves as late as the beginning of July. All strains except strain 1 may have caused a slight stunting but it was impossible to be sure of this point. The buds in the vicinity of strain 3 inoculations were somewhat slow in opening.

On Black Tartarian cherry.—Symptoms on Black Tartarian were, if anything, less conspicuous than on Napoleon. Strain 3 was almost completely masked, while the markings of strain 1, if present at all, were faint on early leaves. With strain 2 the coarse-ring, line, and oak-leaf patterns were few and mild though apparent on new leaves until July (Fig. 3, A). Strain 4 symptoms were much the same as, though somewhat plainer than, those of 2 and persisted on the older leaves for some time (Fig. 3, B).

On Black Eagle cherry.—Black Eagle, inoculated with strains 1, 3, or 4 exhibited a faint mottle early in the spring, and with strain 2 some rings and coarse lines, but when indexed on peach and Mahaleb seedlings was itself

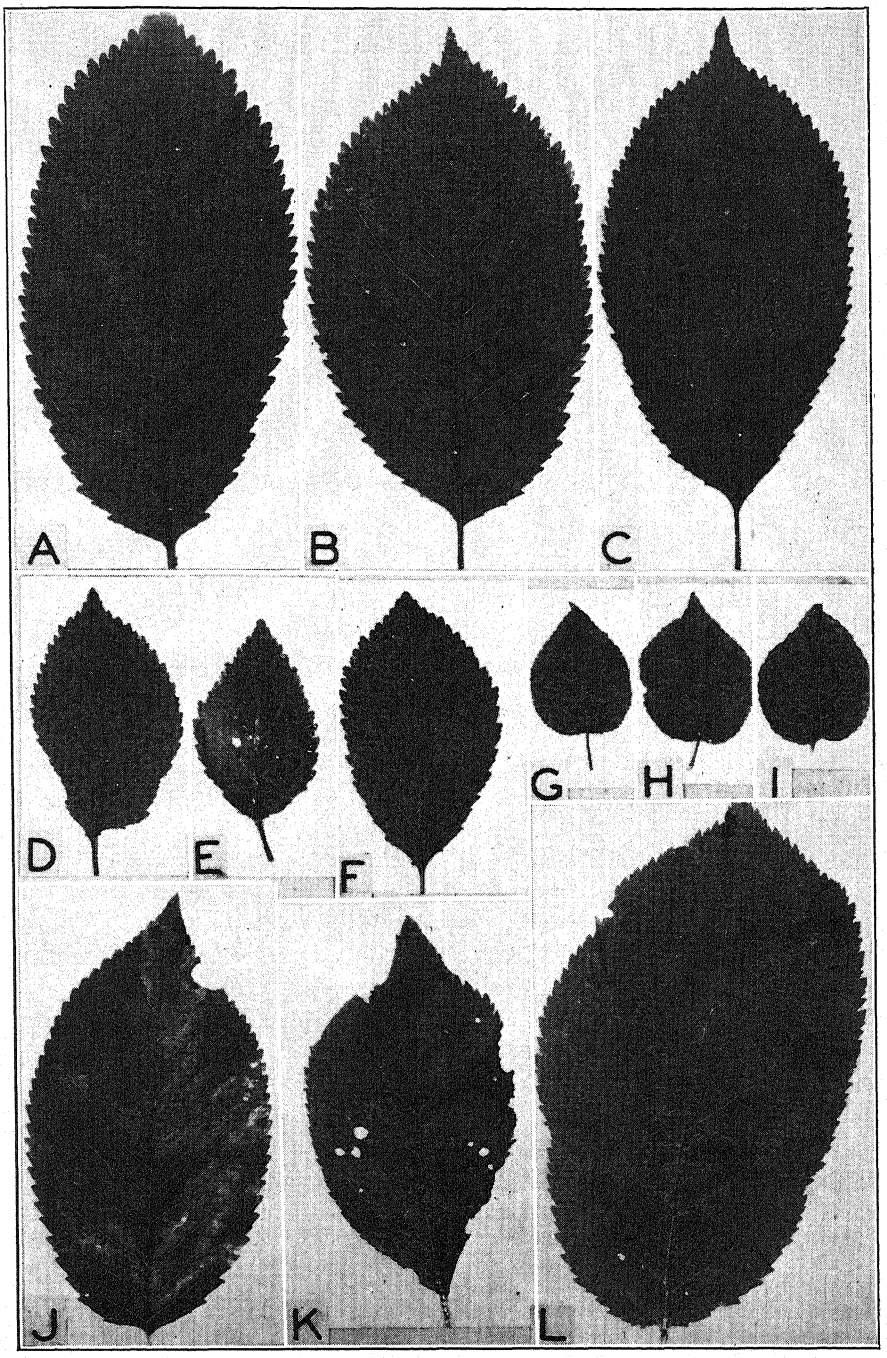


FIG. 3. A: strain 2 and (B) strain 4 on Black Tartarian; C: strain 4 on Napoleon; D–F: strain 2 in Montmorency, D: mottle and rings, E: brown lines and rings, F: brown flecking; G–I: typical symptoms on Mahaleb, G: lines and small rings, H: coarse rings, I: vein-banding; J: symptoms on Elkhorn source of strain 3; K: strain 2 on German prune; L: strain 2 on Grand Duke.

shown to be already virus-infected. The leaf markings on Eagle shoots, however, were in all probability induced by the prune dwarf virus as they were of the same order as those on the other sweet cherry varieties and appeared only on inoculated shoots.

The peach seedlings used for indexing this Black Eagle clon showed both fine and coarse rings and oak-leaf patterns, but the most striking symptom appeared in June as a necrotic streak down one side of each main shoot, near the tip, which was bent over to form a crook. Later the whole tip died. Indexed Mahaleb seedlings were somewhat dwarfed and their leaves were marked with faint oak-leaf and coarse ring patterns in mid-season. The identity of this virus is yet to be established, but it has none of the usual earmarks of prune dwarf.

On Elkhorn cherry.—This variety had either no symptoms or a faint mottle on early leaves. Unfortunately, indexing demonstrated that this supposedly healthy Elkhorn was already carrying a virus presumed to be prune dwarf virus on the basis of the symptoms on indexed peach. If this diagnosis is correct, the virtual absence of symptoms in the inoculated Elkhorn series is understandable since the tree, from which the buds were taken, had been selected as healthy and therefore either had always been symptomless or had long recovered from the initial shock.

On Niagara apricot.—There were no characteristic leaf symptoms of diagnostic value on apricot although, on some of the shoots inoculated with strain 2 or strain 3, some leaves had faint mottling or chlorotic spotting or streaking in the early part of the season. All strains and more especially 1 and 2 induced delay in foliation. Reduction in vigor and growth was considerable with strains 2 and 4 and somewhat less with 1 and 3. As a matter of interest and possibly of significance, two of the three apricot shoots inoculated with strain 2 died during the summer. The fact that, barring two buds that failed to take in other series, these were the only cases of mortality in the apricot experiment suggests that the more virulent strain 2 was responsible. A similar lethal effect with strain 2 has already been mentioned in connection with the double-budding experiment with Italian prune.

On Myrobalan plum.—Myrobalan seedlings exhibited no definite symptoms except with strain 2 when chlorotic mottles, lines, and rings appeared occasionally on early leaves and some plants were more or less stunted. Prune dwarf virus, however, can be transmitted through Myrobalan.

On Mahaleb cherry.—Mahaleb seedlings were quite variable in their reaction. Some were almost symptomless and some had early leaves marked with mottles, lines (Fig. 3, G), coarse rings (Fig. 3, H), and occasionally vein-banding (Fig. 3, I). With strain 2 and to a lesser extent with strains 1 and 3, coarse rings and oak-leaf patterns also appeared on leaves developed late in the summer.

DISCUSSION

In view of the striking similarity of the symptoms of these four viroses on each of the hosts which manifest them, and particularly on Italian prune,

Lombard plum, and peach, little argument is necessary to justify the thesis already proposed that all four are caused by strains of one virus. As a matter of fact, if any distinctions are to be made, there are greater differences between the two strains from plum than between the plum and the cherry strains. Prune dwarf, whether the causal strains of virus be considered individually or collectively, is manifested by symptoms of one or more of the following categories: (a) stunting or reduction of vigor; (b) leaf distortion; (c) patterns on leaves; and (d) crop reduction. The present experiments are not yet of sufficient duration to provide many yield data, but Hildebrand (8, 14) has presented some evidence on that point. With regard to the other types of symptoms, however, the combination exhibited has been much more specific to the host than to the strain.

It should also be observed that, on virtually every host in the range of these experiments, the strains if arranged according to increasing severity of effect would fall in the same order, namely: 3, 1, 4, and 2. Consequently differences occurring between strains, for example, the pattern-forming capacity so pronounced in 2, may be ascribed to differences in virulence. The markings on some hosts, especially with strain 2, bear some resemblance to those induced by the virus of line pattern (1, 3), but some transmission experiments with Shiro plum, which is one of the best indicators of line pattern (1, 9) and with line pattern on the host range, failed to show that that virosis is related to prune dwarf though its virus may be involved as a contaminant in strain 2.

Each species of *Prunus* and, in some instances, each variety has its characteristic reaction to prune dwarf. The typical response of peaches is some degree of dwarfing usually by internode shortening, mild leaf distortion in the form of wavy margins, and foliage patterns of varying intensities and persistence. In cherries, leaf patterns are almost exclusively predominant, at least as primary symptoms, and there is good evidence that some varieties of sweet cherries can become symptomless, or nearly so, with the possible exception of crop reduction. For that matter, *P. mahaleb* and, to a lesser degree, both sweet and sour cherries are not satisfactory differential hosts for prune dwarf because they respond to a number of distinct viroses with confusingly similar markings. Plums have so wide a range of reaction that they can be divided into at least three groups: (a) severely affected varieties, such as Italian prune and Lombard, in which leaf distortion and stunting of growth are the most striking symptoms; (b) moderately or slightly affected varieties, such as Reine Claude, German prune, and Grand Duke, which show almost no leaf distortion and little stunting but some leaf marking; and (c) varieties, such as Abundance and Damson, in which symptoms rarely occur.

The fact that some symptoms, especially leaf patterns, tend to occur only early in the season suggests that the degree of symptom expression may be affected by temperature or other conditions during the period of leaf formation. On the other hand, recovery in the second season would indicate

that, with some hosts at least, infection has an initial shock effect which is not sustained. Both factors are probably operative, though it is not yet possible to assess their relative significance.

The findings recorded here with respect to the effect of prune dwarf on German prune do not agree with those of Hildebrand (8). This discrepancy may have arisen because of differences in the strains of virus employed, but both Hildebrand's failure to obtain symptoms on cherries (8) and his description of peach symptoms would suggest that he was working with a comparatively mild strain and the mild strains in Ontario had little effect on German prune. So it would appear either that different strains of that variety were used in the two investigations or that there was some confusion regarding the identity of German prune in New York State and in Ontario. If either is true and because the same sort of situation might easily arise with other host varieties, stone fruit virus investigations would benefit from some form of standardization of experimental varietal material.

The similarity of the symptoms of prune dwarf on peach to those of rosette mosaic (2, 4, 7) has prompted Hildebrand (7, 8) to suggest a possible relationship between the two viruses. While conclusive proof is yet to be established, it is interesting to note another point of resemblance in that Cation (4) reported transmission of a disease which he diagnosed as rosette mosaic from Damson to peach. The demonstration of strain differences in both diseases opens up the question still further. Whether or not the virus of rosette mosaic is a strain of prune dwarf virus, the natural occurrence of masked forms of prune dwarf on Damson and its presence in sweet cherry twice and probably three times observed serve to indicate that this disease is more wide spread than has yet been realized.

The author wishes to express his sincere thanks to Dr. G. H. Berkeley for his unfailing interest and many helpful suggestions in connection with this investigation.

SUMMARY

Four viroses have been studied in Ontario, two on plum and two on sweet cherry. Symptomatological evidence is presented that the viruses are strains of prune dwarf virus (Prunus virus 6; *Nanus pruni* H.), differing in virulence. All four strains have been transmitted to at least four varieties of *Prunus domestica,* one of *P. salicina,* two of *P. persica,* one of *P. cerasus,* two of *P. avium,* one of *P. armeniaca, P. cerasifera* (Myrobalan), and *P. mahaleb.*

All strains produced typical strap-shaped, rugose, thickened leaves and dwarf growth on Italian prune and Lombard plum; stunting and leaf patterns on peach varieties; and ring, line, and oak-leaf patterns on cherries and *P. mahaleb.* Some plum varieties, namely Reine Claude, German prune, and Grand Duke, showed leaf markings without distortion while some were almost symptomless. Myrobalan was symptomless except with the most virulent strain which induced chlorotic mottles, lines and rings, and sometimes dwarfing.

A tendency to recovery was noted in peaches and cherry varieties after the initial shock symptoms had appeared.

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PHYSIOLOGIC SPECIALIZATION AND THE CONTROL OF MILLET SMUT[1]

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INTRODUCTION

Smut, caused by *Ustilago crameri* Kcke., is one of the important diseases of millet, *Setaria italica* (L.) Beauv., and is coextensive with the cultivation of the crop. In China, millet is one of the principal food crops, the annual production being approximately 28,675,548,000 pounds (1), and the losses caused by the smut are often of considerable economic importance. Reductions in yield of from 10 to 50 per cent in Northern and Northeastern China are not uncommon (6).

Although millet smut can be controlled effectively by proper seed treatment, obviously it would be more desirable and economical to control it by means of resistant varieties. Most of the common varieties of millet so far tested are susceptible; therefore, selection of lines or the breeding of varieties with suitable agronomic characters and resistance to smut would be necessary. As an aid to the breeding program, it is important to learn the number and geographic distribution of physiologic races of the smut fungus and their pathogenicity on the most important varieties. While the ultimate control of millet smut by breeding resistant varieties is being sought, any control which can give immediate relief, such as seed treatment, also is worthy of attention. Therefore, the relative efficacy of new and old seed disinfectants for the control of millet smut was investigated.

PHYSIOLOGIC SPECIALIZATION IN USTILAGO CRAMERI

Physiologic specialization in certain smut fungi has been known for a long time (2, 9), although it has not been recorded for *Ustilago crameri*. It seemed advisable to determine the reactions of the common varieties of millet to various collections of the millet smut.

Varieties of millet were obtained from the Department of Agronomy, College of Agriculture, Honan University, Honan, China, and from the Division of Agronomy and Plant Genetics, University Farm, St. Paul, Minnesota. All the varieties except German No. 18, German Dark, and Siberian No. 13020, were selfed for several years prior to these tests.

Seed lots were soaked for 8 minutes in 1:250 solution of formaldehyde, covered for 4 hours, washed in water for 4 hours, and then allowed to dry thoroughly. The seed was inoculated by applying an equal amount of dry chlamydospores to each lot of seed, about 0.5 g. of smut spores to 100 g. of seed.

[1] A portion of a thesis presented to the Graduate School, University of Minnesota, June, 1937, in partial fulfillment of the requirements for degree of Doctor of Philosophy. Published as paper 2169 of the Journal Series of the Minnesota Agricultural Experiment Station.

During the winter of 1935, preliminary pathogenicity tests were made in the greenhouse with 5 collections of *Ustilago crameri* on 4 varieties of millet. Although the number of plants inoculated was small, the results indicated clearly that the 5 collections of smut differed in their virulence on 4 varieties of millet. On the basis of these preliminary results, more extensive varietal tests were made with more collections of smut.

In the summer of 1935, 11 varieties of millet were inoculated with 9 collections of *Ustilago crameri*, which were obtained from different parts of China. All the varieties of millet tested were planted at University Farm,

TABLE 1.—*Percentage of smutted heads in 12 varieties of millet inoculated with 9 collections of Ustilago crameri, grown in St. Paul, Minnesota, in 1935 and 1936*

Variety of millet	Year	Percentage of smutted heads[a] with different smut collections									
		5	6	8	9	10	12	13	14	17	Control
German No. 10	1935	0	0	0	0	0	0	19	47	3	0
	1936	0	0	0	0	2	0	4	37	10	0
German No. 18	1935	60	51	52	79	73	71	85	63	55	0
	1936	68	35	47	81	42	65	66	39	70	0
German Dark	1935	33	62	42	90	83	88	24	64	63	0
	1936	47	73	38	82	91	77	40	66	76	0
Hungarian No. 232	1935	50	75	59	86	89	81	52	93	81	0
	1936	57	67	53	77	70	64	68	82	57	0
Kaifeng No. 142	1935	63	73	57	61	83	98	51	100	92	0
	1936	67	84	66	69	68	91	70	87	58	0
Kaifeng No. 232	1935	26	51	33	58	65	0	39	0	0	0
	1936	39	69	21	56	77	2	32	0	0	0
Nanking No. 31	1935	5	7	0	0	1	0	16	14	1	0
	1936	0	13	0	0	16	8	43	6	6	0
Teipien No. 48	1935	44	65	58	56	71	75	40	79	39	0
	1936	57	58	16	35	60	57	44	61	48	0
Unnamed Selection No. 11	1935	83	79	75	86	90	89	57	93	96	0
	1936	68	78	58	53	86	76	59	44	83	0
Unnamed Selection No. 12	1935	48	63	34	59	77	87	8	54	36	0
	1936	74	87	43	64	62	72	26	50	30	0
Siberian No. 1120	1935	68	90	77	82	87	84	60	76	53	0
	1936	63	73	66	70	69	49	50	61	55	1
Siberian No. 13020	1936	6	10	40	2	17	58	31	27	26	0
Race No.		1	1	2	1	1	3	4	5	6	

[a] Percentage computed on number of heads in duplicate rows. (The number of heads in each row ranged from 300 to 400.)

St. Paul, Minnesota, in duplicate 5-foot rows, one foot apart, in a split-plot arrangement. The smut percentages were based on counts of 300 to 400 heads in a row. In 1936 similar tests were made, including an additional variety. The results for the two years' tests are summarized in table 1.

There are striking differences in pathogenicity among the collections of smut. For instance, collection 14 produced no smut on the variety Kaifeng No. 232, but more than 85 per cent infection on the variety Kaifeng No. 142; whereas collection 10 of *Ustilago crameri* caused between 65 and 83 per cent infection on these two varieties.

In general, the pathogenicity of a collection on a particular variety proved to be similar in 1935 and in 1936. Fluctuation in the percentages of smut from one season to another is to be expected, because environmental factors may influence profoundly the development of smuts. It is possible that certain collections of smut consisted of many biotypes that differed greatly in parasitic capabilities and in response to environmental conditions.

In a given year all varieties of millet were grown under parallel conditions; hence differences in their reaction to a smut collection must be attributed to differences in virulence of the latter. This certainly would be true of the test made in 1936, as the inoculum and seed sown were produced and stored under identical conditions and the seed was sown the same day in the experimental plots at St. Paul, Minnesota.

Since each collection of smut in 1936 differed somewhat in parasitism, each one might be considered a physiologic race. This fine distinction seems undesirable and also impractical, however, because of the wide fluctuation in percentages of smut that occurred on the same varieties in different years. Therefore, the determination of races was based on fairly wide differences in pathogenicity.

There are at least 6 distinct parasitic races of *Ustilago crameri* (Table 1). Collections 5, 6, 9, and 10 are classified as race 1, because of their similarity in virulence on the 5 differential hosts: German No. 10, Kaifeng No. 232, Nanking No. 31, Unnamed Selection No. 12, and Siberian No. 13020. Each of the other 5 collections consisted of a different physiologic race. Although the separation of collections into races was more or less arbitrary, consideration was given to variation in smut percentages produced by the same races on a given variety in different replications and in different years. The important point is that there are many races of *Ustilago crameri* that must be considered in breeding smut resistant varieties of millet.

Of course, it is possible that, if further tests were made, certain collections that now appear distinct would be grouped together; also it is possible that certain collections might consist of two or more races. Therefore, the separation of collections into races must be considered of a temporary nature.

All varieties of millet tested were susceptible to certain races of *Ustilago crameri* (Table 1). Although a few varieties were highly resistant to several of the races, most of them were susceptible to 3 or more races. German No. 10 was the most resistant variety tested, while Hungarian No. 232, Siberian No. 1120, and German No. 18 were among the most susceptible varieties and were attacked by all the races.

EFFECTIVENESS OF VARIOUS FUNGICIDES IN CONTROLLING MILLET SMUT

The earliest experiments on the control of millet smut by various fungicides were made by Hecke (3, 4). He used copper sulphate, sulphuric acid, mercury bichloride, and formalin; and, in addition, a hot water treatment. Subsequently, many fungicides were tested by several workers (5, 7, 8). The most promising ones were copper carbonate, formaldehyde dip, and

copper sulphate. New Improved Ceresan, a good fungicide for many cereal smuts, apparently has not been tried.

In studying the effectiveness of various fungicides in controlling millet smut, a moderately susceptible variety, Kaifeng No. 232, which had been selfed for 3 years, was used. The inoculum was a mixture of chlamydospores of the 10 collections of *Ustilago crameri*. Prior to treatment, the millet seeds were cleaned and then artificially inoculated with a mixture of chlamydospores of *Ustilago crameri*. The various fungicides and the rate of application are given in table 2. For each treatment, except formaldehyde solution, the amount of fungicide required was first put in a flask and shaken thoroughly; then the seed was added and the flask was shaken again, until the dust covered the seeds thoroughly. When formaldehyde solution

TABLE 2.—*Results of treating Kaifeng millet with 10 fungicides when seed was inoculated artificially with chlamydospores of 10 collections of Ustilago crameri, in 1935 and 1936*

Fungicide		Year and percentage of infection[b]	
Name	Ounces per bushel	1935	1936
New Improved Ceresan	0.5	0.8	0.4
Formaldehyde dip[a]	.....	1.0	1.1
Cuprocide	2.0	3.6	1.0
Copper carbonate (20 pct.)	4.0	3.1	3.1
Barbak III	2.0	.....	4.6
Kopper's flotation sulphur dust	2.0	5.0	10.9
Powdered sulphur	4.0	.....	15.8
Gas sulphur	2.0	16.6	14.2
Sulphur dust	4.0	23.6	18.8
Formaldehyde dust	3.0	29.7	39.7
Powdered sulphur	2.5	36.2	32.2
Check	0.0	26.9	42.9

[a] The concentration used was 1 part formaldehyde to 250 parts of water.
[b] Results were based on 400–500 heads grown in quadruplicate plots. The minimum level for statistical significance between any two means was 0.74 in 1935 and 1.45 in 1936.

was used, the seed was soaked in the solution for 7 minutes, covered for 5 hours, and then washed and dried in the usual manner.

Nine fungicides were tested in 1935, eleven in 1936. In both seasons, the tests were in triplicate, 10-foot-row plots, replicated 4 times and randomized. The percentages of smut were based on the number of heads infected. The results are in table 2.

In 1935 all fungicides used, except powdered sulphur used at the rate 2.5 oz. per bushel and formaldehyde dust, tended to reduce the percentage of infection, but none of them eliminated smut completely. New Improved Ceresan and formaldehyde dip were more effective than the others, although several other fungicides also were partially effective.

The results in 1936 were similar to those in 1935, except for slight differences. There was no apparent stimulation or increase of the percentage of infection when powdered sulphur and formaldehyde dust were used.

In order to determine the statistical significance of the effectiveness of the different fungicides tested, the data for 1935 and 1936 (Table 2) were analyzed separately by the analysis of variance method. The minimum level for statistical significance between any two means was 0.74 in 1935 and 1.45 in 1936. New Improved Ceresan and formaldehyde dip were the most effective fungicides in 1935; cuprocide and copper carbonate were next. Kopper's flotation sulphur dust was more effective than any other sulphur fungicides.

DISCUSSION

Millet is a food crop which is exceeded in importance in Northern China only by wheat. Smut caused by *Ustilago crameri* is one of the most important diseases of millet. Because of the importance of the crop and the losses caused by this smut, its control is of major importance. While it can be fairly well controlled by certain seed treatments, the development of resistant varieties will be very important in China, where it often is difficult to induce farmers to treat the seed.

Because of the importance of physiologic races of smuts in breeding disease resistant varieties of cereals, the writer investigated the question of physiologic specialization in *Ustilago crameri*. In 1935 eleven varieties of millet were inoculated with 9 collections of smut from China. These collections differed greatly in their pathogenicity. In 1936 the tests were repeated, using one additional variety of millet, and similar differences occurred.

The differences in the reactions to the 9 collections in 1935 and 1936 are not unexpected. Chlamydospore collections may contain many biotypes, and, furthermore, recombinations can be expected. Also, it is well known that differences between biotypes or groups of biotypes may become apparent under some conditions and not under others. In spite of the fluctuation in percentages of smut in certain cases, there are at least 6 distinct parasitic races of *Ustilago crameri*. This fact is of great importance in breeding disease resistant varieties. Although there were great differences in reaction of millet varieties to different races, none of those tried was resistant to all the smut races. Consequently further search must be made for resistant varieties.

SUMMARY

Nine collections of *Ustilago crameri* were tested for their virulence on 12 varieties of millet. Of these, 6 differed sufficiently in pathogenicity on 5 differential hosts to be designated as physiologic races.

Although certain varieties of millet were highly resistant to several races, no variety was resistant to all races.

The prevalence of races of smut must be considered in breeding millet varieties resistant to the disease.

Eleven fungicides were tested as seed disinfectants, and all except powdered sulphur and formaldehyde dust reduced smut. New Improved

Ceresan was very effective; formaldehyde dip, cuprocide, copper carbonate, and Barbak III were fairly effective. In general, sulphur fungicides were not effective.

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PARASITISM OF RHIZOCTONIA SOLANI ON BEANS[1]

L. H. PERSON

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In most parts of the United States, and especially in the South where moisture and temperature conditions are favorable, many species of plants are attacked by fungi belonging to the genus *Rhizoctonia*. While these fungi are a heterogeneous group, many of them, because of certain sclerotial characteristics, are considered by most investigators to be *Rhizoctonia solani* Kühn.

That *R. solani* comprises distinct strains differing in morphology, cultural characters, and pathogenicity has been shown by many investigators. Among them are Peltier (12), Rosenbaum and Shapovalov (14), Edson and Shapovalov (2), Matsumoto (9), van der Meer (10), Walker (18), Lauritzen (7), Wiant (19), Gratz (6), Elmer (3), LeClerg (8), Briton-Jones (1), Monteith and Dahl (11), and Sanford (15). The more recent studies of LeClerg (8) and Sanford (17), in which large numbers of isolates from Irish potatoes were tested for pathogenicity on sugar beets, indicated that nearly all were nonpathogenic on this host. These results are of particular interest when certain control problems, such as crop rotation, are being considered.

In Louisiana the common garden bush snapbean, *Phaseolus vulgaris* L., is often attacked by *Rhizoctonia*. Stands are reduced and reddish-brown lesions develop on the stems near the soil surface. As these fungi occur on many hosts other than beans in this State, it seemed desirable to determine if isolates from other hosts were pathogenic on beans.

To obtain this information, a number of isolates were secured from various hosts in Louisiana and from other investigators. Some studies were made of the cultural characters and reactions to temperature of the various cultures, but the major part of the investigations was devoted to a study of their pathogenicity on beans.

ORIGIN OF ISOLATES

From a rather large number of isolates, 27 were finally selected for these studies. Pertinent information regarding them is in table 1.

CULTURAL CHARACTERS

The isolates were compared on bean and potato-dextrose agar. Differences occurred in such characters as the color of the mycelium, rate of growth, and sclerotium formation. A representative group of 6 isolates is shown in figure 1.

[1] Summary of part of a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted by the University of Minnesota, August, 1937.

EFFECT OF TEMPERATURE ON RATE OF GROWTH

Twelve isolates from 8 hosts differed considerably in rate of growth on potato-dextrose agar in Petri dishes at 7 different temperatures (Table 2). Only a trace of growth occurred at 6° C. in the isolates from tomato (R7), eggplant (R6), and bean (R76), as compared to 9 to 17 mm. with the other 9 cultures. The optimum temperatures also differed, being from 20° to

TABLE 1.—*Origin of isolates of Rhizoctonia solani*

Number of isolate	Host plant	Part of plant from which isolated	Location	By whom isolated	Date of isolation
R3	Sugar cane	Root	Baton Rouge, La.	E. C. Tims	1932
R4	do	do	do	do	1932
R5	do	do	do	do	1932
R6	Eggplant	Stem base	do	S. Smith	1933
R7	Tomato	do	do	do	1933
R8	Potato	do	do	do	1933
R9	Bean	do	Houma, La.	L. H. Person	1931
R62	Sugar beet	—	Chaska, Minn.	E. L. LeClerg	1930
R64	Bean	Stem base	Houma, La.	L. H. Person	1931
R76	do	do	Baton Rouge, La.	do	1931
R77	do	do	Houma, La.	do	1932
R100	Sugar beet	Seedling	Ohio	E. L. LeClerg	1930
R110	Potato	Tuber (sclerotium)	St. Paul, Minn.	do	1930
R115	do	Stem	Guthrie, Minn.	do	1931
R120	Pea	Root	St. Paul, Minn.	G. H. Starr	—
R127	Potato	Stem	Dilworth, Minn.	E. L. LeClerg	1931
R128	do	do	do	do	1931
R150	Potato	Tuber (sclerotium)	Amherst, Mass.	do	1933
R162	do	do do	Scotts Bluff, Neb.	do	1933
R170	do	do do	Hay Springs, Neb.	do	1933
R178	do	— —	California	do	1933
R188	Rice	Seedling	Welch, La.	D. E. Ellis	1932
R191	Potato	— —	Topeka, Kansas	E. L. LeClerg	1933
R194	Rice	Seedling	Welch, La.	D. E. Ellis	1932
R200	Potato	Tuber (sclerotium)	Kentville, Nova Scotia	E. L. LeClerg	1933
R208	Sugar cane	Stalk (interior)	Baton Rouge, La.	E. C. Tims	1934
R310	Pea	— —	Moscow, Idaho	W. H. Pierce	1935

25° C. for the isolate from potato (R8) and from 25° to 30° C. for the others. Isolates from sugar cane (R4), bean (R9), and sugar beet (R100) grew slowly at 38° C., while the others did not grow.

METHODS USED IN PATHOGENICITY TESTS

For the pathogenicity tests, isolates were grown on a sterile mixture of 4 parts oats and 1 part wheat to which water was added at the rate of 80 per cent by weight. The cultures used for inoculum usually were from 4 to 6 days old when the inoculations were made.

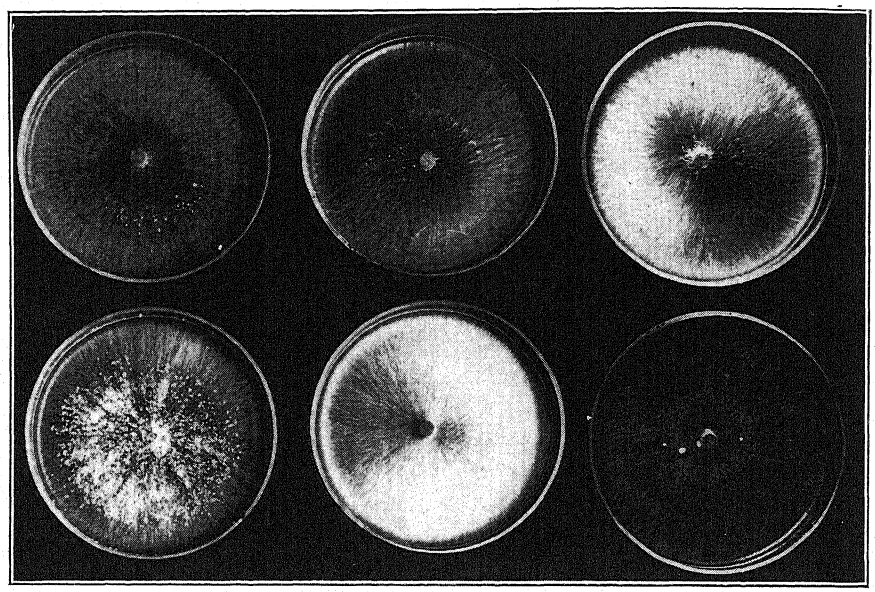


FIG. 1. Six culturally different isolates of *Rhizoctonia solani*.

Inoculations were made in the greenhouse in sterilized soil and in the field. The greenhouse inoculations were of two types: soil inoculation and direct seedling inoculation. The soil was inoculated by mixing approximately 25 grams of the inoculum into 6-inch pots of sterilized soil. After 4 to 6 days the soil was remixed and the seeds were planted one inch deep. Bean[2] seedlings were inoculated directly by removing the soil from the base of the stem and placing the inoculum against the basal-stem portion. The soil was then replaced, covering the inoculum.

Seedlings were inoculated directly in the field similarly to those in the

TABLE 2.—*Effect of temperature on the rate of growth of 12 isolates of Rhizoctonia solani on bean agar (average of 3 tests of 3 replications each)*

Original host	Isolate number	Average diameter of colonies in mm. after 48 hours						
		6° C.	15° C.	20° C.	25° C.	30° C.	34° C.	38° C.
Sugar cane	R4	10	22	37	61	62	54	8
Potato	R8	14	26	36	37	22	11	0
Rice	R194	14	26	43	80	78	69	0
Pea	R120	17	35	51	71	54	23	0
Pea	R310	16	31	49	73	52	26	0
Bean	R9	10	30	47	80	78	57	8
Bean	R64	9	30	51	86	85	43	0
Bean	R76	1	32	50	85	79	48	0
Tomato	R7	1	26	40	69	75	64	0
Eggplant	R6	1	30	47	87	83	66	0
Sugar beet	R62	12	21	35	52	43	23	0
Sugar beet	R100	11	22	39	65	59	43	10

[2] Unless otherwise stated, the term bean refers to bunch snapbeans (*Phaseolus vulgaris*).

greenhouse. For the soil inoculations in the field, the wheat-oats inoculum was mixed with sterilized soil at the rate of 2 quarts to 4 gallons of soil. After two weeks this was mixed thoroughly and dropped into open furrows, 30 grams every 8 inches. The seeds were then dropped into the inoculated hills and covered immediately.

To determine the pathogenicity of the isolates, two methods were used. In one, the percentage stand was used. In the other, an arbitrary scale of infection was used, with 5 classes ranging from 0 to 4. The plants were removed from the soil, washed, and placed in one of the five following infection classes:

0 = No stem lesions (no infection).
1 = One to few small shallow lesions (light infection).
2 = One or several lesions extending into the cortex (moderate infection).
3 = One or more lesions extending deep into the cortex, often completely girdling the stem (severe infection).
4 = Plants killed.

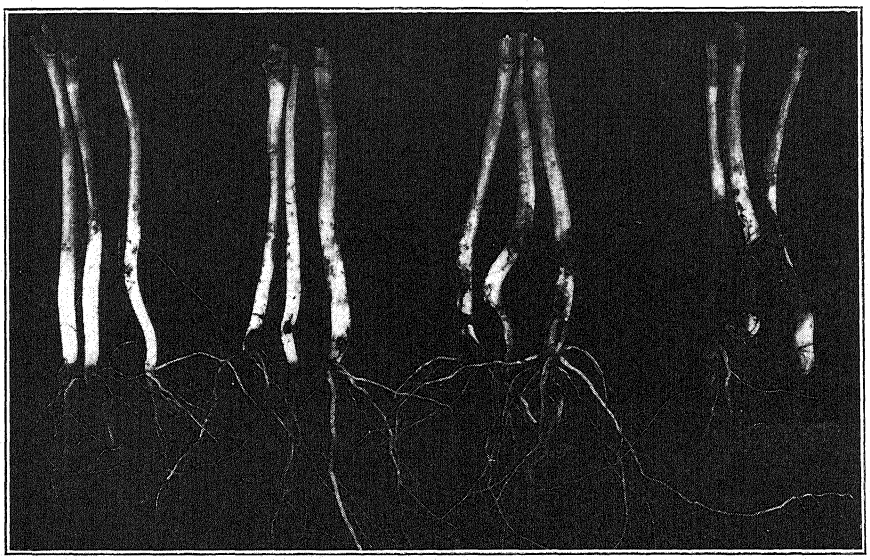


FIG. 2. Infection classes of bean plants inoculated with *Rhizoctonia solani*. Left to right, classes 0, 1, 2, 3.

The average degree of infection for an isolate was then computed by adding the infection class values of individual plants and dividing by the total number of plants. The infection classes 0, 1, 2, and 3 are shown in figure 2.

WOUNDS AND THE PATHOGENICITY OF RHIZOCTONIA ON BEANS

The first experiment, using 24 isolates from 7 hosts, was made in sterilized soil in the greenhouse to learn if wounded bean seedlings were more

TABLE 3.—*Results of inoculating wounded and nonwounded bean plants with 26 isolates of Rhizoctonia solani*

Original host of isolate	Isolates used	Plants not injured		Plants injured	
		No. inoculated	Degree of infection	No. inoculated	Degree of infection
Sugar cane	R3, 4, 5	27	0.00	27	0.00
Potato	R8, 110, 115, 127, 128, 150, 162, 170, 178, 191, 200	102	0.00	98	0.002
Rice	R188, 194	15	0.87	19	1.94
Pea	R120, 310	30	1.46	26	1.88
Bean	R9, 64, 76, 77	37	3.37	38	3.53
Tomato	R7	9	3.44	10	3.60
Eggplant	R6	8	3.63	10	4.00
Averages			1.81		2.14
Control		10	0.00	10	0.00

severely attacked than nonwounded ones. Young plants were wounded by slitting the basal stem portions with a scalpel and an equal number were left unwounded for comparison. The inoculum was placed directly against the stems of injured and noninjured seedlings and covered with soil. Wounding had relatively little effect on the severity of parisitism: the average degree of infection on nonwounded plants was 1.81 and on wounded plants 2.14 (Table 3). The 3 sugar-cane isolates and 11 Irish potato isolates were not pathogenic on either wounded or nonwounded bean plants. The

TABLE 4.—*The degree of infection on 3 varieties of beans inoculated with 14 isolates of R. solani in the field at St. Paul, Minn., 1934*

Original host	Isolate number	Degree of infection[a]			
		Giant Stringless	Bountiful	Webber Wax	Average for 3 varieties
Sugar cane	R4	0.30	0.40	0.50	0.40
Potato	R8	0.15	0.35	0.40	0.30
	R110	0.20	0.70	0.35	0.41
Rice	R194	0.80	0.70	0.85	0.78
Pea	R120	1.35	1.15	1.30	1.27
	R310	1.30	1.05	1.40	1.25
Bean	R9	2.75	2.70	2.70	2.72
	R64	3.25	3.35	3.25	3.28
	R76	2.95	3.15	3.05	3.05
	R77	2.85	2.70	3.25	2.93
Tomato	R7	2.95	3.00	3.00	2.98
Eggplant	R6	2.05	2.45	3.05	2.52
Sugar beet	R62	3.30	3.30	3.45	3.35
	R100	2.95	2.90	3.10	2.98
Control		0.10	0.15	0.25	0.17

[a] Average of 2 tests of 3 replications each.

others differed in pathogenicity, ranging from an average degree of infection of 1.41 for the rice isolate (R194) to 3.83 for the eggplant isolate (R6). The wounded plants wilted 1 to 2 days earlier than the nonwounded ones. This agrees with the results of Fulton (5), who reported that Rhizoctonia-infected noninjured plants wilted as frequently but not so quickly as slightly injured plants.



FIG. 3. Beans inoculated with *Rhizoctonia solani* in the field at St. Paul, Minn. The row on extreme left is a check. The 2 center rows from left to right were inoculated with isolates R76 and R64, respectively. The row on extreme right was inoculated with isolate R110.

PATHOGENICITY OF RHIZOCTONIA ON BEANS IN THE FIELD

Seeds of 3 varieties of beans, the Giant Stringless, Bountiful, and Webber Wax, were planted in the field at St. Paul, Minn., and, after emergence, the seedlings were inoculated directly. The soil was removed from around the stems of the plant and the inoculum applied, after which it was covered with soil. The results (Table 4) were essentially the same as in the earlier test in sterilized soil. The sugar-cane isolate (R4) and the 2

potato isolates (R8 and R110) were not pathogenic. The rice isolate (R194) was slightly pathogenic; the pea isolates (R120 and R310) were somewhat more pathogenic than the rice isolate; and the four from bean, the tomato, the eggplant, and 2 sugar-beet isolates were very pathogenic to all 3 bean varieties. This difference in pathogenicity is shown in figure 3. A similar test was made at Baton Rouge with the Giant Stringless, Bountiful, and Sure Crop Wax varieties. The results were essentially the same.

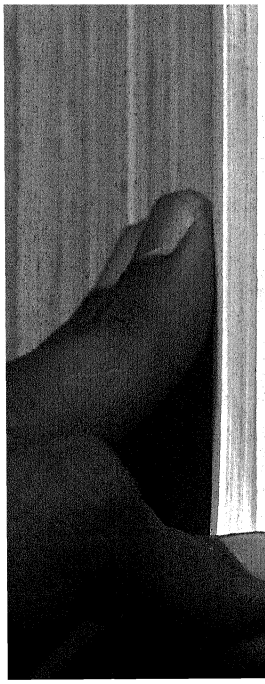
In 2 tests with the same varieties of beans and with the same isolates, the soil was inoculated and the bean seed planted immediately. The percentage stand of beans in the soil inoculated with each isolate is given in table 5. It may be seen that the isolates again differed in their patho-

TABLE 5.—*Percentage stand of 3 varieties of beans in field soil inoculated with 14 isolates of Rhizoctonia solani at St. Paul, Minn.*

Original host	Isolate number	Stand in per cent[a]		
		Giant Stringless	Bounti-ful	Webber Wax
Sugar cane	R4	91	89	88
Potato	R8	92	92	86
	R110	89	89	85
Rice	R194	16	21	10
Pea	R120	82	92	85
	R310	92	89	86
Bean	R9	92	90	82
	R64	86	86	77
	R76	89	89	79
	R77	92	89	83
Tomato	R7	87	85	84
Eggplant	R6	86	84	76
Sugar beet	R62	54	68	47
	R100	56	62	36
Control	.....	92	93	86

[a] Average of 3 replications of 104 seeds each.

genicity. The rice isolate (R194) was the most pathogenic of all isolates, decreasing stands of all three varieties. It had been only moderately pathogenic, however, when the size of lesions on the stem was used as a criterion (Table 4). The sugar-beet isolates (R62 and R100) were next in pathogenicity, reducing stands considerably. The sugar-cane (R4) and potato (R8 and R110) isolates did not reduce emergence. An analysis of variance using the method given by Fisher (4) was also made of these data. Highly significant differences (exceeding 1 per cent point) existed in the relative pathogenicity of the isolates and the reactions of the three varieties to the isolates.



PATHOGENICITY OF RHIZOCTONIA ON BEANS AND OTHER LEGUMES IN THE GREENHOUSE

Beans.—Experiments in sterilized soil in the greenhouse under more rigidly controlled conditions also served to test the various isolates as to their

pathogenicity on beans. The results with 25 isolates from 8 hosts are in table 6. The 3 sugar-cane isolates, the 11 potato isolates, and the 2 pea isolates had no effect on the stand of beans. The rice isolate (R194), as in previous tests, almost completely prevented emergence of the bean seedlings. The 4 bean isolates, the tomato isolate, the eggplant isolate, and 2 sugar-beet isolates reduced stands considerably.

Other Legumes.—As the field and greenhouse tests indicated that the various isolates could be separated into 4 groups, based on their patho-

TABLE 6.—*Percentage stand of beans in sterilized soil inoculated with 25 isolates of Rhizoctonia solani*

Original host	Isolate number	Number of tests	Number of seeds	Stand in per cent
Sugar cane	R3	1	24	88
	R4	2	124	87
	R5	2	124	95
Potato	R8	4	324	90
	R110	4	324	94
	R115	3	224	92
	R127	3	224	89
	R128	3	224	90
	R150	3	224	93
	R162	3	224	90
	R170	3	224	89
	R178	3	224	90
	R191	3	224	88
	R200	1	24	96
Rice	R194	2	124	5
Pea	R120	2	124	94
	R310	2	124	92
Bean	R9	2	124	65
	R64	2	124	76
	R76	4	324	52
	R77	2	124	52
Tomato	R7	2	124	50
Eggplant	R6	2	124	39
Sugar beet	R62	2	124	46
	R100	1	100	64
Control	.....	4	324	89

genicity on beans, further experiments were made with several additional species of plants. The tests were in sterilized soil in the greenhouse and the hosts were soybeans, English peas, cowpeas, broadbeans, and beans. Thirteen isolates from 8 hosts were used. The results (Table 7) indicate that the sugar-cane isolate (R4) and the potato isolates (R8 and R110) which had been nonpathogenic on beans were not pathogenic on soybeans (*Soja maxima* L.), English peas (*Pisum sativum* L.), cowpeas (*Vigna sinensis* Endl.), and broadbeans (*Vicia faba* L.). The rice isolate (R194), which was highly pathogenic on snapbeans, also was very pathogenic on English peas, cowpeas, and broadbeans, but was not so pathogenic on soybeans (34 per cent stand as compared to 49 per cent stand in control). The 2 pea isolates (R120 and R310), which were equally pathogenic on beans

and reduced stands very little, differed in their effects on the other hosts. R310 was somewhat pathogenic on soybeans, while R120 was not. On English peas and broadbeans R310 was much more pathogenic, almost completely preventing emergence. On cowpeas there was little difference between the 2 isolates, both reducing stands somewhat. The 3 bean isolates (R9, R64, and R77), the tomato isolate (R7), the eggplant isolate (R6), and the 2 sugar-beet isolates (R62 and R100), in previous tests on beans, had about the same pathogenicity range, reducing stands somewhat; but they differed on soybeans, English peas, and cowpeas, the sugar-beet isolates being less pathogenic on soybeans and English peas than the others, and

TABLE 7.—*Percentage stands of 5 leguminous hosts in sterilized soil inoculated with 13 isolates of Rhizoctonia solani*

Original host	Isolate number	Stand in per cent[a]				
		Bean	Soybean	English pea	Cowpea	Broadbean
Sugar cane	R4	93	51	90	91	80
Potato	R8	88	47	85	89	70
	R110	94	49	92	93	90
Rice	R194	4	34	12	1	0
Pea	R120	83	44	60	76	90
	R310	82	24	1	69	0
Bean	R9	52	1	2	28	0
	R64	78	13	3	27	0
	R77	56	8	1	28	0
Tomato	R7	46	5	3	47	0
Eggplant	R6	61	19	1	9	0
Sugar beet	R62	57	31	68	93	0
	R100	58	41	54	91	0
Control	.....	93	49	93	91	80

[a] Figures for broadbean were obtained in one test; those for cowpea are averages of 2 tests; those for other crops are averages of 3 tests.

nonpathogenic on cowpeas. The tomato isolate could possibly be distinguished from the bean and eggplant isolates in that it was less pathogenic than they were on cowpeas. All isolates from bean, tomato, eggplant, and sugar beets prevented emergence of broadbeans.

EFFECT OF ENVIRONMENT ON PATHOGENICITY

To determine the effect of temperature on the pathogenicity of the isolates on beans, seeds were planted in inoculated sterilized soil held at various temperatures. Seven isolates were tested and the percentage stand of beans was used as the criterion of pathogenicity. The isolates were pathogenic from 15° to 28° C., being most pathogenic at the lowest temperature (Table 8). These results agree with those of Richards (13) and Sanford (16). The potato isolate (R8) used was not pathogenic at any of the 3 temperatures.

A test was then made to determine the effect of soil moisture on the emergence of bean seedlings in soil inoculated with various isolates of *R. solani*.

TABLE 8.—*Effect of soil temperature on the pathogenicity of 7 isolates of Rhizoctonia solani on beans*

Original host	Isolate number	Stand in per cent[a]			
		15° C.	20° C.	24° C.	28° C.
Potato	R8	68	78	.....	60
Control	.....	70	75	.....	70
Rice	R194	7	2	0	.....
Bean	R9	0	0	.....	25
	R64	64	76	73	.....
	R76	25	53	45	55
	R77	47	53	60	.....
Sugar beet	R62	7	13	11	.....
Control	.....	76	78	89	80

[a] Based on 40 seeds at each temperature for each isolate.

Seven isolates and 3 moisture contents were used. Stands were reduced about equally at the 3 soil moistures but the average degree of infection was more severe at 60 and 80 per cent soil moisture than at 40 per cent (Table 9).

EFFECT OF AMOUNT OF INOCULUM ON PATHOGENICITY

Isolate R194 from rice was so pathogenic on beans when seeds were planted in inoculated soil, almost preventing emergence in the various tests, that a special study was made to determine if the amount of inoculum was responsible for the severity of its attack.

In 4 experiments the amount of inoculum was varied. In 2 tests the amount of oat-wheat inoculum varied from 50 g. to 2 g. per 6-inch pot. Seeds were planted in half the pots immediately after inoculation and in the other half 4 days later. In the other two tests, 25 g. of inoculum were added to each 6-inch pot. After 4 days the inoculated soil was removed and thoroughly mixed. This mixture was then added to sterilized soil in the following proportions by weight: 1:0, 3:1, 1:1, 1:3, 1:7, and 1:15.

TABLE 9.—*Effect of soil moisture on the pathogenicity of 7 isolates of Rhizoctonia solani on beans*

Original host	Isolate number	Moisture content in per cent					
		40	60	80	40	60	80
		Stand in per cent[a]			Degree of infection		
Potato	R8	83	93	60	0.00	0.04	0.00
Rice	R194	0	0	0	.....	.....	.....
Pea	R310	92	88	67	0.64	0.38	1.05
Bean	R9	63	52	25	0.34	1.87	1.20
	R76	88	68	28	0.58	1.71	1.41
Eggplant	R6	65	40	47	0.95	1.38	1.54
Sugar beet	R100	65	58	48	0.46	0.80	1.17
Control	.....	99	90	65	0.00	0.00	0.00

[a] Based on 60 seeds for each isolate at each temperature.

TABLE 10.—*Results of soil inoculations with different amounts of inoculum of isolate R194 showing percentage emergence of beans*

Time of planting seed	Emergence in per cent[a]						
	Amount of inoculum (in grams) added to each 6-in. pot of soil						
	50	32	16	8	4	2	Control
Four days after soil inoculation	0	2	0	3	0	0	94
At time of soil inoculation	0	0	0	0	0	0	98
	Ratio of inoculated to noninoculated soil						
	1:0	3:1	1:1	1:3	1:7	1:15	Control
Three days after soil inoculation	0	0	0	0	0	0	93
At time of soil inoculation	5	4	2	5	4	1	95

[a] Based on 5 replications of 20 seeds each.

The same procedure was followed in planting, half the pots being planted at the time of inoculation and the remainder 3 days later. The results (Table 10) show that neither the amount of inoculum nor the time of planting had any effect on the degree to which the fungus prevented emergence.

PARASITISM OF ISOLATE R194 ON BEAN SEEDLINGS

A test was made using 12 pots of soil inoculated with cultures of R194 and 12 pots, as controls, in which only the sterile oat-wheat mixture was

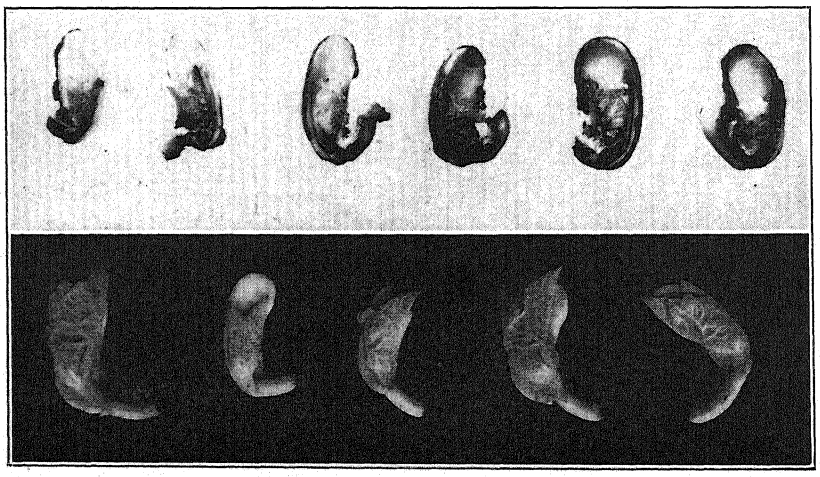


FIG. 4. Effect of *Rhizoctonia solani* isolate R194 on bean seed. Top: Seed planted in inoculated soil. Bottom: Seed planted in sterilized soil.

added to the soil. Fifteen bean seeds were planted in each pot and 4 days later the seeds were removed from 1 pot of inoculated soil and 1 pot of control soil. In the control soil the 15 seeds had germinated while in the inoculated pot 4 seeds had germinated. The seed coats from the inoculated soil were covered and penetrated by the mycelium of the fungus and the radicles were attacked where they emerged from the cotyledons (Fig. 4). Two days later another inoculated and another control pot were examined. All 15 seeds had germinated in the control, and 10 in the inoculated soil. Only a slight elongation of the hypocotyl, however, had taken place in plants from the inoculated soil. Each hypocotyl was infected at the point of emergence from the seed and the plumular leaves were dark brown. Rhizoctonia mycelium occurred in the plumules. Eight and 10 days after planting, inoculated and control pots were again examined and the seedlings were similar to those previously examined. In the 6 inoculated pots remaining, 26 days after planting, no seedlings had emerged, while 82 healthy plants were in the 6 control pots.

DISCUSSION AND SUMMARY

Rhizoctonia solani can cause damping-off and stem rot of beans under a rather wide range of temperature and soil moisture content. The isolates studied comprise a number of strains differing in cultural characters and rate of growth at different temperatures.

When beans were used as the test host, 4 rather clear-cut groups of isolates were distinguished on the basis of pathogenicity: (1) isolates from sugar cane and from sclerotia found on Irish potato tubers, which were not pathogenic on beans; (2) isolates from peas, which caused only a very slight amount of damping-off and only a moderate stem infection; (3) the isolate from rice, which almost completely prevented emergence but caused less severe stem lesions than the pea isolates; (4) isolates from bean, tomato, eggplant, and sugar beet, which were capable of reducing stands somewhat and caused very severe lesions on stems. The isolates in groups (2) and (4) could be further subdivided when cowpeas, English peas, soybeans, and broadbeans were added as differential hosts.

The nonpathogenicity of the sugar-cane and potato isolates is of interest and may be of possible importance when considering the rotation system in the alluvial sections of Louisiana. These hosts are important economic crops in some of these areas.

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SAPSTREAK, A NEW KILLING DISEASE OF SUGAR MAPLE

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INTRODUCTION

In the fall of 1939, attention was called to an unusually heavy mortality among large sugar maples (*Acer saccharum* Marsh.) in the Big Ivy section of the Pisgah National Forest, near Barnardsville, N. C. Local residents reported this dying to have been taking place since about 1935. The Big Ivy working circle of the forest supports, in part, a stand of old-growth northern hardwoods, including about 4 million board feet of virgin sugar maple of very high quality. In the mountains of North Carolina, as a whole, the Forest Survey of the U. S. Forest Service estimates that there are approximately 54 million board feet of sugar-maple sawtimber.

Surveys by Mr. A. H. Maxwell, then with the Pisgah National Forest, and the author, indicated that the new disease, sapstreak, was restricted to a few neighboring creek drainages. About 60 trees were dead or dying along Corner Rock Creek, and about a dozen more were scattered in the other drainages. As of 1943, the disease seems to be confined to the area delimited by these drainages, although sugar maple occurs abundantly over wide areas contiguous to the affected areas.

SYMPTOMS

Ordinarily 3 or 4 years elapse between the first crown symptoms and death of a tree from sapstreak. In occasional cases, affected trees died suddenly when in full leaf. Although the development of the disease has not been followed in detail, certain of its characteristics are obvious and outstanding. The first external symptom is usually a dwarfing of the foliage to about half normal leaf size, causing the crowns to look thin. A slight chlorosis usually accompanies this dwarfing. The next year the upper part of the crown starts to die (Figs. 1, A, and 2, A), and within another year or two the entire tree may die. One large tree was dead within a year of the time the first thinning of the foliage was noted, while other trees have been known to remain alive 4 years after first showing symptoms.

The trunk wood becomes watersoaked in wide radial streaks from the inner sapwood into the outer sapwood, sometimes to the cambium, looking much like figure 1, C, of the paper by Davidson and Campbell (3) on a gall of sugar maple. This watersoaking approaches nearest the cambium in the lower part of the trunk. There are often green streaks at the margins of the watersoaked areas, and in the middle of the watersoaked "fingers" are always narrow streaks, reddish or gray when the wood is moist and gray

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when it is dry. The watersoaking and the gray streaks in the trunk always accompany decline of the crowns. Wherever the watersoaking reaches the cambium, this tissue is killed at the points of contact. In the few cases in which entire trees were cut into sections for examination, the watersoaking had developed from the base of the tree upward, and disappeared at a height of 20 to 40 feet.

Sapstreak has not been found in trees under 8 inches in diameter, at breast height, and no intermixed red maples (*Acer rubrum* L.) have been found affected. The roots of one large dying sugar maple were excavated. Portions of the root system were dead, as was to be expected in trees of great

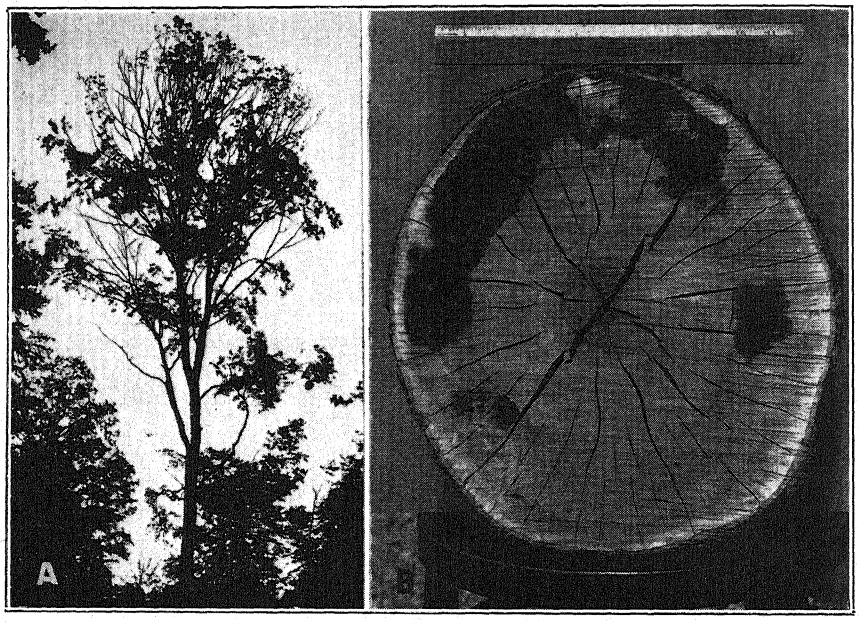


FIG. 1. A. Naturally infected sugar maple showing dying of the upper crown, a typical early external symptom of the sapstreak disease. B. Section from a naturally infected trunk 37 feet above ground, showing the discoloration by *Endoconidiophora virescens*.

age. Gray streaks and watersoaked areas were abundant in some of the larger roots, and seemed to have developed where large roots had died. Rhizomorphs and mycelial fans of *Armillaria mellea* were abundant on the dead roots, a condition common to the roots of many hardwoods of advanced age in this region. A *Xylaria* was also associated with some of the dead roots, and *Ustulina vulgaris* was causing some of the decay in dead roots.

ISOLATIONS

About 200 isolations, on both malt and Bacto-peptone agars, have been made from various tissues. Only one organism, *Endoconidiophora virescens* Davidson, has been consistently isolated from the discolored wood of the trunk. *E. virescens* had been included under *E. coerulescens* until David-

son, in a recent paper (2), recognized and described this American fungus as distinct from the European *E. coerulescens*. The maple sapstreak fungus was identified both by R. W. Davidson and C. T. Rumbold, and has been previously reported only as a cause of blue-stain in hardwood logs and lumber, and occasionally behind injuries in both hardwoods and softwoods.

Cultures prepared from the watersoaked or green-streaked tissue surrounding the reddish or grayish radial streaks generally yielded either no organisms or occasional bacteria of different kinds. Almost invariably, however, when the implanted tissue included a bit of a gray or reddish streak, the culture yielded *E. virescens*. This indicates that we may regard the watersoaking and green streaks as host responses to the presence of the fungus in certain adjacent elements.

E. virescens has been consistently isolated from the internal streaks in a large number of diseased trees. In one tree the highest sapwood discoloration, at a height of 40 feet, occupied less than half the circumference of the sapwood and the inner bark was alive; and the fungus was isolated. The discoloration and watersoaking in the first 10 feet of the trunk involved the entire circumference of the outer sapwood, and all of the inner bark to this height was dead and brittle. The foliage of the tree had died a few months previously. Figure 1, B, shows a section taken at a height of 37 feet that was set face down on the laboratory floor for 4 days to bring out the infection pattern of the fungus. The internal nature of the infection can plainly be seen since a band of uninvaded normal sapwood separates the stained portions from the cambium almost all the way around.

The pH of sugar maple wood is normally in the vicinity of 5.5. Freshly cut wood in the watersoaked areas, had a pH of 8.5 or above, as determined by the Hellige Triplex indicator applied directly to the wood.

INOCULATIONS

Since *E. virescens* was generally associated with the trunk symptoms, inoculations were made in the stems and roots of sugar maples, using single-spore cultures of this fungus. The trees inoculated in April, 1940, ranged from 17 to 34 inches in diameter at breast height, and were in the locality where other trees had been diseased, although no trees had died of sapstreak on the small tract selected.

The fungus was grown on rye and introduced into trees by the following methods: (1) in 3 bore holes about 3 inches deep made with a $\frac{3}{8}$-inch auger, arranged more or less equidistant around a tree at breast height, (2) in 1 bore hole in each of 2 roots near the base of a tree, (3) in a pocket about 6×6 inches gouged out of the stem at breast height, holding the inoculum in the pocket by a large rubber patch, (4) in similar pockets gouged out of 2 roots. Three trees were inoculated by each method, and, using sterile rye, one check tree was prepared for each method.

Table 1 gives the results of these inoculations, and figure 2 shows two of the inoculated trees 3 years after inoculation.

TABLE 1.—*Results of inoculation of sugar maple with Endoconidiophora virescens*

Inoculation method	Total trees	After 2 years			After 3 years			
		Normal	Diseased	Dead	Normal	Diseased	Dead	Total infected
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Stem								
Bore holes	3	2	1	0	2	0	1	1
Poultices	3	3	0	0	2	1	0	1
Checks	2	2	0	0	2	0	0	0
Root								
Bore holes	3	2	1	0	1	2	0	2
Poultices	3	0	2	1	1	1	1	2
Checks	2	2	0	0	2	0	0	0
All inoculated	12	7	4	1	6	4	2	6
All checks	4	4	0	0	4	0	0	0

Both stem and root inoculations, using either the bore-hole or poultice method, resulted in extensive infection in one or more trees. At the end of 3 years, of 12 trees inoculated, 2 were dead, and 4 others were obviously diseased. Figure 2, A, shows a tree inoculated by means of a stem poultice. Its crown has become very thin, at the end of the third year, and the tree will doubtless die within a year. Figure 2, B, shows a tree that was inoculated



FIG. 2. A. An 18-inch sugar maple inoculated by stem poultice 3 years before this photograph was taken. The crown is still alive but has thinned considerably. B. A 20-inch tree inoculated through three auger holes 3 years earlier. This tree was a perfect specimen when inoculated and was dead 3 years later.

through three bore holes. It was a vigorous tree when inoculated, had dwarfed foliage after 2 years, and was dead at the end of 3 years. Figure 3, A, shows the streaking that developed from one of the bore holes in the tree shown in figure 2, B, by the end of the second year, while the tree was still alive.

None of the check trees, representing all types of inoculation, but receiving sterile rye as inoculum, have become diseased, nor have any of the many untreated sugar maples among the treated trees on this tract become diseased.

Endoconidiophora virescens has been reisolated from the streaks in the three inoculated trees cut into for this purpose. The results of the inoculations have been striking enough to warrant careful study of the disease, including inoculation experiments with small saplings, in which the development of the pathogen can be more readily followed.

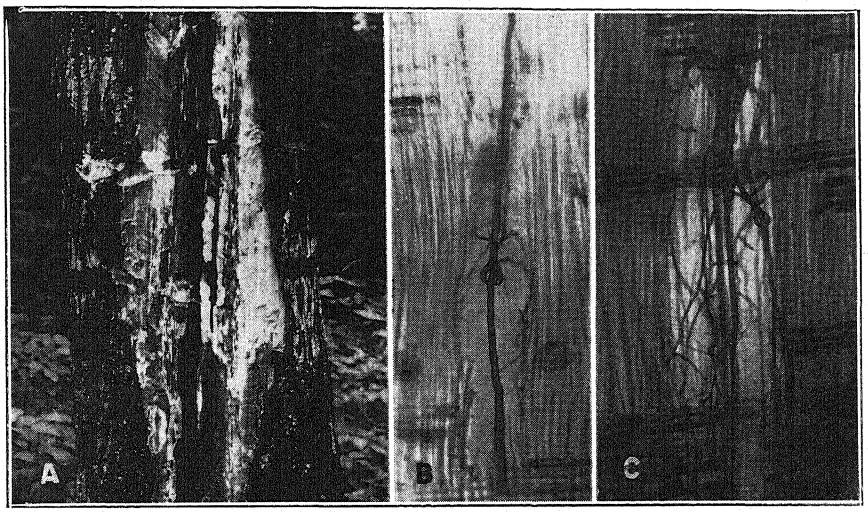


FIG. 3. A. Extensive streaking of the sapwood of the tree shown in figure 2, B, two years after inoculation. The inoculation hole is in the center of the picture. B. Large hypha of *Endoconidiophora virescens* in a vessel, bearing a lyre joint. C. Mass of hyphae in vessels, one of which bears a lyre joint.

From the manner in which the gray streaks radiate outward, it appears as though the fungus establishes itself in the inner sapwood of the trunk, possibly coming up from dead roots, and works outward. Death of the surrounding ray and wood parenchyma cells by toxin production or breakdown of conduction by invaded elements may cause the watersoaking, and the occasional green streaks seem to be a host reaction common in living maple wood induced by the presence of certain organisms. Green streaks also occur with the maple wilt *Verticillium,* and have been found in other cases of organic irritation of maple sapwood (3).

THE CAUSAL FUNGUS

Endoconidiophora virescens, under the name *E. coerulescens,* has been reported as one of the most prevalent fungi causing sap stain in hardwood

logs and lumber in the United States (1, 2, 5, 6), occurring also occasionally on pine. That a fungus with such a habitat should also be able to develop luxuriantly in the living sapwood of a vigorous sugar maple, spreading rapidly through this tissue and killing the tree, is most unusual. Lagerberg, Lundberg, and Melin (4) describe spread of the closely related *E. coerulescens* from blazes made on living spruces, to over 10 feet longitudinally and 1.3 inches in depth, in 20 months. Even in these cases, however, there was no general spread through the sapwood, such as takes place with *E. virescens* in sugar maple. Some members of the closely allied genus *Ceratostomella* are active parasites, such as *C. ulmi* and the fungus that causes the canker-stain disease of the plane tree (7). Neither of these fungi, however, generally occur as sap-stainers of logs and lumber. Culturally the fungus causing sapstreak of sugar maple agrees closely in color, odor, and morphology, with *E. virescens* isolated from stained yellow-poplar logs and lumber.

TABLE 2.—*Comparison of isolates of Endoconidiophora virescens after 14 days in Petri plates of malt agar*

Characteristic	Isolate number[a]			
	1	2	3	4
Sweet, musty odor	Strong	Mild	Strong	Strong
Perithecia	Rudiments	None	Abundant	Few
Sphaeroid endoconidia	Abundant	None	Abundant	Abundant
Rod-shaped endoconidia	Abundant	Mod. abundant	Abundant	Abundant
Growth rate[b]	Rapid	Slow	Rapid	Moderate

[a] Culture 1—From sap stain in yellow poplar log.
" 2—From inoculated sugar maple. Deteriorated culture.
" 3—From surface lumber stain, yellow poplar. Fresh isolate.
" 4—From naturally diseased sugar maple. Fresh culture.

[b] Slow —Colony 9 centimeters in diameter after 7 days at room temperature.
Moderate— " " " " " " 6 " " " " " .
Rapid — " " " " " " 4 " " " " " .

Both fungi are highly variable in culture, especially old cultures. A culture of the sugar-maple sapstreak fungus isolated from an inoculated tree grew profusely in culture when first isolated, had a strong, sweetish, musty odor, and sporulated abundantly. After a year in the refrigerator, fresh agar plantings grew much slower, had less odor, and sporulated much less. Davidson (2) mentions variability and sectoring in *E. virescens*. Table 2 compares four isolates, two from diseased sugar maple and two from yellow poplar sap-stained after felling.

The cultures listed in table 2 have been used in new inoculations to determine whether isolates from sap-stained lumber will cause the sapstreak disease. The determination of the potentialities and means of control of sapstreak will depend to a large extent on whether or not the lumber-stain and sugar-maple sapstreak fungi are identical physiologically.

The hyphae of *E. virescens* are generally confined to the vessels, and in this way differ sharply from the conifer sap-stain Ceratostomellae that normally live in the rays. Hyphae of the sapstreak fungus are brown and

develop copiously in the vessels and rarely in the rays. The larger hyphae branch peculiarly at some of the septa, often producing a structure in the shape of a lyre, for which the name "lyre joint" is proposed (Fig. 3, B and C). Lyre joints form only at a septum. One side branch or two opposite side branches may form. These branches are usually short, and come to a tapering point. Sometimes they grow long and may branch farther. Each side branch bears a septum just above the point of anastomosis with the main hypha. The two opposite branches bending inward toward the main hypha just beyond the point of anastomosis give them a lyre-like appearance, but sometimes they are not incurved. Lyre joints have been found abundantly both in diseased maple wood and in sap-stained yellow-poplar lumber. A search for similar structures in wood stained by other fungi is being conducted. Lagerberg, Lundberg, and Melin (4) do not mention the occurrence of lyre joints in their description of the closely allied *E. coerulescens,* nor have they been reported for other fungi.

SIGNIFICANCE OF THE DISEASE

A close watch is being kept on the development of the disease in the affected area. Notes were taken in 1941 on the condition of 6 trees, along the truck trail through the area, that showed slight evidence of crown thinning. In 1943, two of these were dead, the others had become much worse, and some new cases could be seen from the road.

A 13-acre plot containing 78 large sugar maples, two of which were diseased, was established in the affected area in 1941. In 1943, the two diseased trees were dead, and one additional tree had crown symptoms.

Any disease that works so rapidly that a 20-inch tree can be killed in 3 years by inoculations in three small holes in the trunk, must be considered potentially dangerous. A large volume of valuable sawtimber in the Big Ivy section is already diseased. It remains to be seen whether or not the disease is increasing in amount from year to year in the affected area, and if it is spreading to other areas. The Forest Service is harvesting the diseased timber now before it dies and every effort is being made to salvage as much of this material as possible.

SUMMARY

A disease of sugar maple, for which the name "sapstreak" is proposed, has been killing large numbers of trees in a part of the Pisgah National Forest, near Asheville, N. C.

Healthy sapwood of vigorous trees is invaded by a strain of the fungus *Endoconidiophora virescens,* which produces gray to reddish radial streaks and watersoaked areas in the wood. The foliage becomes progressively smaller and paler green each year, and death usually occurs within 2 to 4 years from the first evidence of crown-thinning.

Inoculation of 12 large trees with *E. virescens* resulted in 2 dying and 4 becoming heavily diseased, in 3 years.

The causal fungus is morphologically indistinguishable from the common sapstain fungus on hardwood lumber, earlier referred to *E. coerulescens*, and recently renamed *E. virescens*.

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MACROPHOMINA ROOT AND STEM ROT AND ANTHRACNOSE OF CHAMAECRISTA[1]

J. L. WEIMER

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Chamaecrista procumbens (L.) Greene and *C. fasciculata* (Michx.) Greene, commonly known as partridge pea or sensitive pea, are low, woody annuals that grow wild along highways and in waste places in many parts of southeastern United States. Since they are able to survive such adverse conditions, these plants have been studied by scientists of the Soil Conservation Service, United States Department of Agriculture, to determine their usefulness in preventing soil erosion. Investigators have found that considerable numbers of plants in Soil Conservation Nurseries and elsewhere are killed by diseases. The present investigations have determined 2 causes of the death of these plants.

In September, 1941, the writer visited the Soil Conservation Nursery at Thorsby, Alabama, to inspect dying plants of *Chamaecrista.* Large plants of both species were being killed: *Chamaecrista procumbens* was affected by a root rot, the cause of which was not immediately evident, whereas *C. fasciculata* was dying from injury to the stem near the surface of the soil produced by the larva of a snout weevil.[2] This insect injury was later found to be prevalent in plants growing along highways in Georgia.

A survey for diseases that might limit the usefulness of the partridge pea in a soil-erosion program has revealed the existence of an anthracnose that is potentially capable of destroying or damaging many plants. The root rot and the anthracnose have been studied and the results obtained are reported in this paper.

ROOT ROT

Symptomatology

A fairly high percentage of plants of *Chamaecrista procumbens* growing in rows in the nursery at Thorsby were dead or dying. The tops of some of the plants were somewhat wilted, others were brown and dead. The plants appeared to have been affected over a considerable period of their life cycle, since there were varying degrees of stunting, the upper nodes often were shortened somewhat, and the leaflets were small, giving the ends of many branches a rosetted appearance and suggesting the presence of a virus (Fig. 1). The roots had a dark-brown rot and some were badly disintegrated. There was no fungus fruiting on the surface and no other clue to the cause of the disease was observed at the time.

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Georgia Agricultural Experiment Station. Paper number 127, Journal Series, Georgia Agricultural Experiment Station.

[2] Determined as *Contrachelus carolinensis* Schoof by H. F. Schoof, Raleigh, North Carolina.

Isolation of the Pathogen

A number of diseased plants with different types of symptoms were taken to the laboratory and several isolations were made from the roots, stems, and twigs. Some plants were placed in a can of water and held at room temperature for about 3 weeks for further observation.

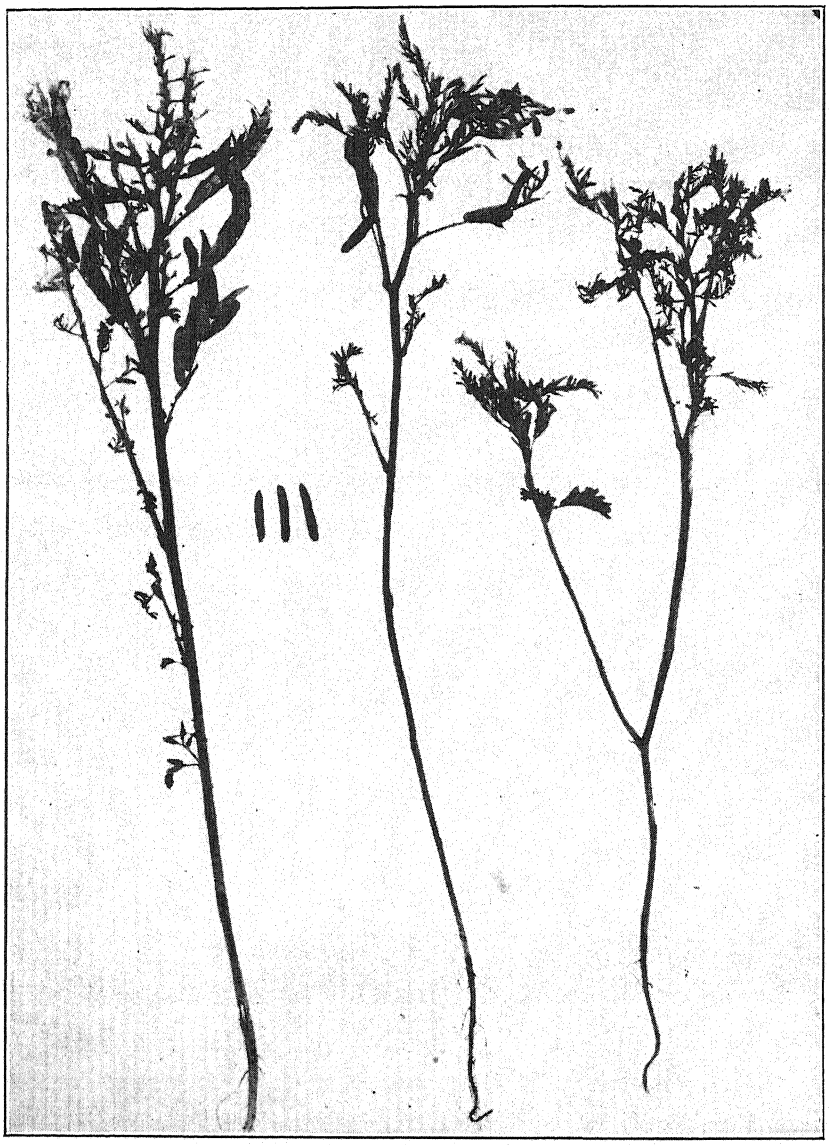


FIG. 1. Plants of *Chamaecrista procumbens* attacked by *Macrophomina phaseoli*. Naturally infected plants from Thorsby, Alabama, with tops greatly stunted and tap and fibrous roots badly decayed. $\times \frac{2}{3}$.

A sclerotium-producing fungus was isolated from 5 of 9 twigs, from 4 of 6 stems, and from 2 of 6 roots. Other fungi were isolated, some of which

failed to fruit and were not identified, and on some plates only *Aspergillus sp.* appeared.

After the plants had been held with their roots in water for a week or longer, many large pycnidia appeared on their stems, usually on the 2½ inches between the top of the water and the top of the container. These pycnidia contained rather large, ovoid, hyaline, unicellular spores.

The fungus was in the tissue of practically all diseased plants and readily produced sclerotia and pycnidia on host tissue and formed sclerotia in culture. It was identified as *Macrophomina phaseoli* (Maub.) Ashby and was the cause of root rot and death of the plants.

The fungus from *Chamaecrista* was compared with isolates from other hosts but no consistent difference was found. A culture from *Chamaecrista* was used by K. H. Garren in his studies on ashy stem blight of beans (1). He found that it attacked beans, and formed the typical pycnidia and spores of *Macrophomina* on the dead bean tissue, thus leaving no doubt of the identity of the organism.

Pathogenicity of Macrophomina

To determine the pathogenicity of this fungus on *Chamaecrista procumbens,* several inoculation experiments were conducted, the seed being supplied by the Soil Conservation Service at Thorsby, Alabama. Seed were immersed for 20 minutes in concentrated, commercial sulphuric acid and then washed in running water for one hour. This treatment served to scarify the seeds and to disinfect their surfaces. The soil, in 6-inch pots, was autoclaved for 3 hours at 25 pounds' pressure, and then held in the greenhouse. After an inch or so of the top soil had been removed from each pot, about 25 seeds were placed on the surface and then covered thinly with part of the soil that had been removed. About 2 tablespoons of inoculum, consisting of sterilized oats on which the fungus was growing, was next placed over the surface of the soil in each pot and the remainder of the soil added. Controls were prepared in the same manner except that sterile oats were used instead of inoculum. The seeds were planted and inoculum was added Nov. 26, 1941, and the final notes were taken Feb. 17, 1942.

Only half as many seedlings appeared in the inoculated as in the control pots because of preemergence decay. The control plants remained healthy, grew to maturity, and set some seed. Some of the seedlings in the inoculated pots were dwarfed very early; others died and pycnidia were formed on the stems just above the soil. Many had very scant fibrous root systems and were dwarfed but lived to maturity. A few plants had pycnidia on the stems when the experiment was discontinued. Isolations were made from several plants and the roots of the remainder were placed in water. The fungus was recovered from each plant from which isolations were made and pycnidia were formed along the base of the stems of the plants placed in water. Hence, all plants in the infested soil contained the fungus. Isolations were made from a number of seeds produced on diseased plants, but the

fungus was never recovered. It is possible, however, that the seed coats were so hard that the fungus could not grow through them.

On July 7, 1942, another experiment was set up in the same manner, except that the fungus used was a reisolate from the previous experiment. In this test there was considerable preemergence decay. Some seedlings died soon after emerging and others grew to be an inch or two tall before they succumbed. Infection seemed to take place at almost any point on the hypocotyl (Fig. 2), the cotyledons, the plumule, or the young roots. As soon

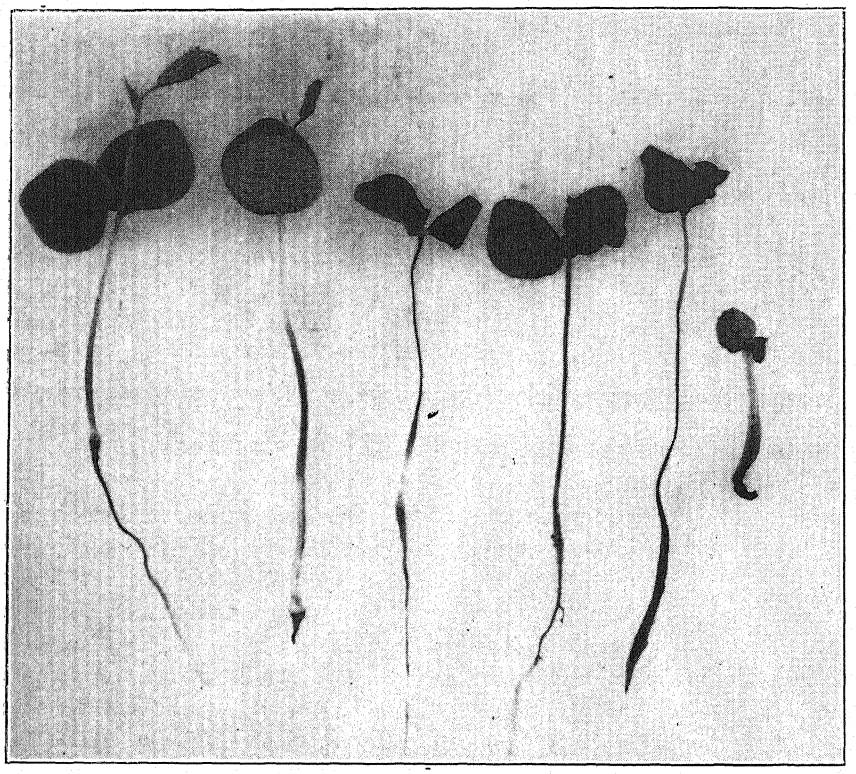


FIG. 2. Seedlings grown in the greenhouse in artificially infested soil. Plant at the left is nearly normal size and has only a slight superficial canker on the hypocotyl near the surface of the soil. The second plant from the left has a slightly deeper lesion at about the same place. The third, fourth, and fifth plants have their hypocotyls almost entirely involved and nearly severed. The hypocotyl of the plant at the extreme right was decayed early and the seedling remained dwarfed. $\times 1\frac{1}{2}$.

as plants were 2 to 3 inches tall, however, they seemed to become resistant to this seedling decay and generally reached maturity. Some plants affected in the seedling stage, but not killed, grew to maturity, whereas the others died before maturity. When the plants approached maturity and had become greatly weakened or had died, many pycnidia appeared on the stems, usually at the base first but often higher, even on the branches. This raised the question as to whether these plants had not become infected until they were nearly dead, or had been infected in the early stages of growth but had not been injured appreciably.

In the hope of obtaining some information on this point, isolations were made from a number of plants that had passed the seedling stage, some being as tall as 12 inches but with little or no evidence of infection, to determine if they were invaded but not injured by the fungus. Some plants selected for this test wilted slightly at times, others were somewhat dwarfed, and still others appeared to be healthy. The plants were disinfected with calcium hypochlorite or alcohol and mercuric chloride, 1–1000, and ½-inch segments were planted on agar in plates. Segments were numbered from the root upwards so that it could be determined how far up the stem the fungus had grown. Sometimes alternate segments were planted. In one experiment 10 plants were used and the fungus was recovered from all, being isolated from the roots of 4 plants and from the stem just above the ground of the other 6. In another experiment the fungus was recovered from 4 of 6 plants. It was isolated from the roots of 3 and from the stem of the fourth plant about 2 inches above ground. In a third experiment the fungus was recovered from the root or crown tissue of 9 of 15 plants. It was obtained from the first above-ground segment as well as from the roots of 6 plants and from all the segments up to 3 inches above ground of one plant.

Forty-two plants growing in infested soil in one experiment grew to maturity and after dying they were examined for the presence of the fungus. Pycnidia were found on the stems of 36 plants and sclerotia were present under the epidermis of the other 6.

ANTHRACNOSE

In the autumn of 1942, plants of *Chamaecrista procumbens* growing under very dry conditions on uncultivated ground at Experiment, Georgia, were attacked by a fungus that killed the ends of the branches for a distance of 2 to 4 inches. The ends of affected twigs, with the brown dead leaves still attached, had fallen over to form a Shepherd's crook near the junction of the dead and live tissue (Fig. 3, A).

Isolation of the Pathogen

Some of the dead stems were held in a moist chamber for 48 hours at which time many salmon-colored spore masses were present on the dead tissue. By touching a sterile needle to the mass of spores and transferring those that adhered to it to a tube of sterile water and then plating loops of this spore suspension, it was possible to get a pure culture of the fungus.

Cultural and microscopic studies of the fungus isolated from the diseased plants have shown no consistent morphological difference between this pathogen and the conidial stage of *Glomerella cingulata* (Ston.) Spauld. and Schrenk from apple. Fifty spores from culture measured 4.5–6 × 10–17 μ (Av. 5.2 × 13.9 μ) which is close to the spore measurements given by Shear and Wood (5). An attempt to infect apple with the fungus obtained from *Chamaecrista* failed. Apples inoculated with the fungus isolated from apple were decayed. Nevertheless, in the absence of any morphological difference

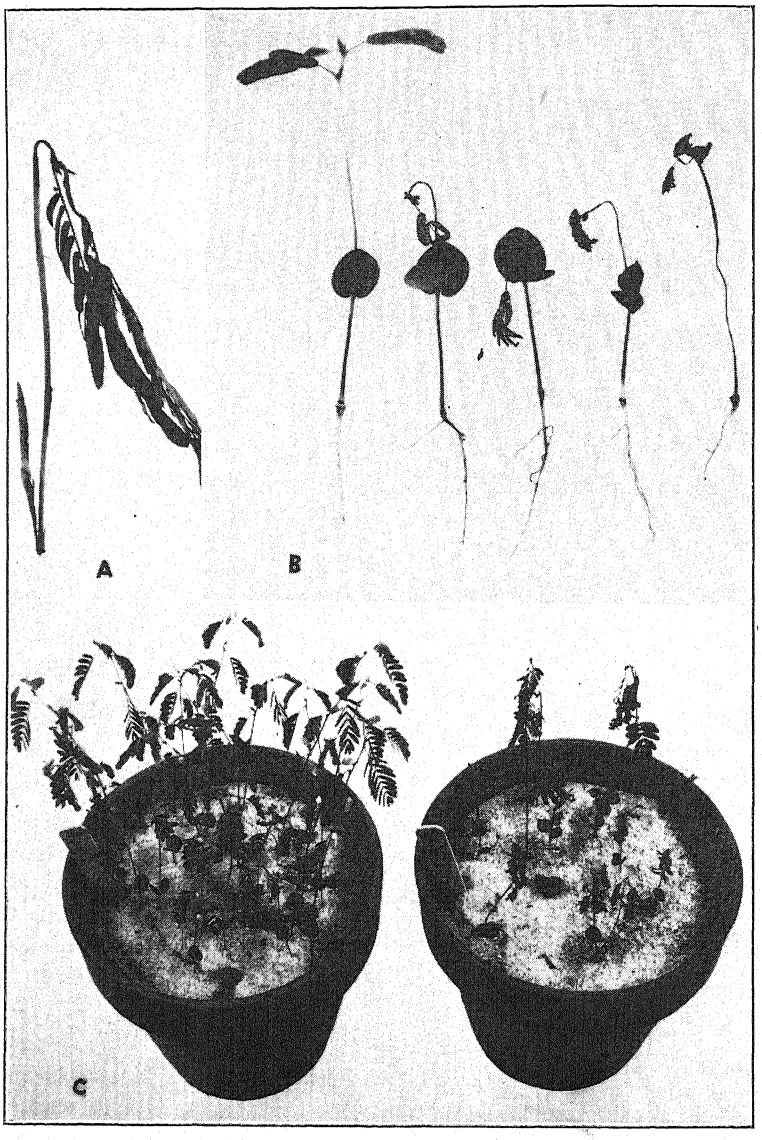


FIG. 3. Plants of *Chamaecrista procumbens* attacked by *Glomerella cingulata*. A. Naturally infected twig. Petiole and adjacent stem were killed and the weight of the leaf has bent the weakened stem into a shepherd's crook. ×1⅓. B. Control plant at the left. The other plants show varying degrees of injury to the stem above the cotyledons. The leaves of the affected plants have collapsed. The two plants at the right have more extensive injury and the stems below the cotyledons have become involved, the cotyledons have shriveled, and in case of the plant at the extreme right, have fallen. Photographed 5 days after inoculation. ×1⅓. C. Two pots of older plants, controls at the left and at the right infected plants with collapsed terminal shoots. Photographed 6 days after inoculation. ×$\frac{2}{10}$.

sufficient to differentiate it from the strain of *G. cingulata* from apple, this fungus from *Chamaecrista* is considered to be a strain of that species.

Pathogenicity of Glomerella Isolates

In the first inoculation experiment 4 pots of seedlings about 2 inches tall growing in sterilized soil in the greenhouse were used. The seed had been scarified and sterilized with sulphuric acid. A heavy suspension of spores was made by adding sterile water to an oat-agar slant on which the fungus was sporulating abundantly. Two pots of plants were atomized with the spore suspension and then placed in a moist chamber with 2 other pots atomized with sterile water as controls. The inoculations were made Nov. 11, 1942, and the pots were left in the moist chamber for 48 hours. There was no evidence of infection at the end of 48 hours, but in 96 hours the inoculated plants showed distinct injury. The petioles were dying and the leaflets wilting. In another day many of the leaflets were dry, the petioles were dead, and some of the cotyledons and stems were decidedly injured. All 15 plants inoculated were diseased, some being entirely dead above ground, others having their leaflets and cotyledons killed but the stem below the cotyledons still alive, and still others having the stem and cotyledons alive but the petioles and leaflets dead. The petioles usually were killed first and the fungus grew from these into the stem which sometimes was killed back only a short distance and at other times to the ground or lower. In general, the cotyledons remained free of infection, but often died as a result of the death of the stem. The appearance of some of the plants 5 days after being inoculated is illustrated (Fig. 3, B). On Nov. 17, 1942, 6 days after the inoculations, all seedlings in one pot and 9 in the other were dead. Of the 6 remaining plants in the second pot, all parts above the cotyledons were dead. All control plants remained healthy. The fungus was readily recovered from the dead seedlings. One week after the inoculations, 5 inoculated plants, whose stems had not been killed down as far as the cotyledons, were still alive. These plants were forming new buds and stems in the axils of the cotyledons. The plants were considerably retarded, however, and never grew as rapidly as the controls. It seems probable that had the pots been held in the moist chamber another 24 hours none of the plants would have survived.

This experiment shows that under high humidity seedlings of *Chamaecrista procumbens* are very susceptible to *Glomerella cingulata*. A reisolate of the fungus was used in other experiments and it also was very pathogenic to seedlings.

Having demonstrated that seedlings are very susceptible, older plants were inoculated to test their susceptibility. In one experiment 3 pots of plants 4 to 5 inches tall were used, the plants in 2 of the pots being inoculated Nov. 24, 1942, and those in the third being held as controls. Four days later all the inoculated plants showed some injury. The petioles were killed just as in seedlings. The stems at the base of the leaf stalks were injured in some cases and the fungus was fruiting abundantly on the dead petioles and stems. A large proportion of the leaflets on plants with 2 leaves were dead, but when there were 3 or more leaves those below the second were still

alive. The controls had 4 to 7 leaves at this time and all were healthy (Fig. 3, C). The fungus seemed to make little further progress and since it was thought that this might be due to the dry atmosphere of the greenhouse, one pot of plants was returned to the moist chamber for 24 hours. No appreciable change took place. On Dec. 9, 1942, one pot had 10 plants with the tops nearly dead; 5 of the plants each had one leaf alive and only the cotyledons remained on the other 5. These plants were so weakened that even though they did not die they always remained greatly stunted. In one instance a new shoot was being formed in the axil of a cotyledon.

Thus, severe injury and often death may result from the disease, even when plants 4 to 5 inches tall are attacked. In another experiment, still older plants were used. These plants were green but vegetative growth had ceased and nearly mature pods were present. No infection was obtained. The results of this experiment confirm previous observations that only actively growing tissue is susceptible, at least under the conditions under which the disease has been studied.

Seedlings of *Chamaecrista fasciculata* were inoculated at the same time with the same inoculum and were held in the same moist chamber as the mature plants just discussed. Abundant infection was obtained. This demonstrated that the inoculum and the environmental conditions were suitable for infection and also that *C. fasciculata* probably is as susceptible to this fungus as is *C. procumbens.*

DISCUSSION

Chamaecrista procumbens, especially in the seedling stage, is very susceptible to *Macrophomina phaseoli,* just as are many other legumes as well as a wide variety of non-leguminous plants (2, 3, 4). Plants infected in the seedling stage, but not killed, may die later or may grow to maturity. In some experiments the plants appeared to become resistant to infection when they were 3 to 4 inches tall. However, these experiments were in the greenhouse with plants growing in pots that dried out at times. This deficiency of water at irregular intervals may have resulted in a more rapid hardening of the stems, thus slowing down or preventing infection, than would have existed in plants of the same age under field conditions. Possibly the drying may affect the pathogenicity of the fungus as well as the resistance of the host. It is known that the pathogenicity of the fungus on the same host is quite variable in different localities. For example, Garren (1) pointed out that in 1941 at Experiment, Georgia, ashy stem blight and southern blight together accounted for less than 5 per cent of the recorded dead bean plants, whereas at Tifton, Georgia, ashy stem blight alone was responsible for 60 per cent of the dead plants. Investigators working on this disease of beans at the Georgia Agricultural Experiment Station have informed the writer that ashy stem blight is always much more destructive at Tifton than at Experiment. The precise cause of the variation in destructiveness of this parasite is unknown, but it is thought to be some environmental factor, probably tem-

perature or moisture, or both (2, 4, 6). It is quite possible that the *Macrophomina* root rot of *Chamaecrista procumbens* may be much more severe in the field at Thorsby, Alabama, or elsewhere, than in the greenhouse at Experiment. This appeared to be the case.

An anthracnose caused by *Glomerella cingulata* was found damaging the terminal shoots of *Chamaecrista procumbens*. Experiments proved that this fungus is very pathogenic on seedlings and on vigorously growing parts of older plants under conditions suitable for infection.

It seems probable, therefore, that either anthracnose or root rot, or both, may cause considerable loss of young plants of *Chamaecrista procumbens* under environmental conditions suitable for the rapid spread and development of the fungi, conditions such as often exist during prolonged wet periods accompanied by high temperatures in late spring or early summer. Considerable damage may be caused by the anthracnose fungus to *C. fasciculata* also.

SUMMARY

Two diseases of *Chamaecrista* have been described, a root rot and an anthracnose caused by *Macrophomina phaseoli* and *Glomerella cingulata*, respectively. So far as known, the former fungus attacks *C. procumbens* only, but the latter is destructive both to this species and to *C. fasciculata*. The root-rot fungus was more pathogenic on young plants under the experimental conditions, but observations indicated that older plants can be killed. The anthracnose fungus can kill seedlings in a few days; only the actively growing ends of the branches of older plants are susceptible, and mature plants are highly resistant.

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